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Application News

Semi-Preparative Supercritical Fluid Chromatography System - Nexera™ UC Prep

# Semi-Preparative Online SFE-SFC for Solid-State Injection and Purification

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## User Benefits

- High throughput and low co-solvent consumption due to the use of subcritical carbon dioxide as the extraction fluid and mobile phase.
- Simplified sample preparation enable by the use of the extraction oven as “solid-state autosampler”.
- Fast and efficient injection and purification of target compounds, even at the gram scale.

## ■ Introduction

Preparative (prep.) supercritical fluid chromatography (SFC) offers significant advantages over prep. liquid chromatography (LC), particularly in terms of efficiency and sustainability. SFC employs pressurized $CO_2$ as the main mobile phase component, reducing solvent consumption and enabling faster separations due to its lower viscosity and higher diffusivity. Since the mobile phase is in a sub/supercritical state, samples cannot be dissolved in it as in liquid chromatography, often causing peak distortion or precipitation. Despite these challenges, liquid injection remains predominant, with solvent selection and injection volume playing a crucial roles in maintaining peak integrity. As an alternative, a supercritical fluid extraction (SFE) oven can be used as “solid-state autosampler”. Using the Nexera UC Prep in semi-prep online SFE-SFC configuration, "solid-state injection“ was performed. In this method, the sample dissolves in the extraction fluid before being transferred to the SFC part of the system, reducing polarity mismatches and minimizing precipitation risks.

In this study, we evaluated key parameters impacting sample loading, including the methanol (MeOH) ratio during extraction and the duration of both static (SE) and dynamic extraction (DE). The optimized method was then applied to a few grams sample, to confirm upscaling possibility.

This work is based on the original article published in Journal of Chromatography Open[1].

## ■ Semi-preparative online SFE-SFC system

The Nexera UC Prep is a compact and versatile system for semi-prep. scale purification (Fig. 1).

Fig. 1 Nexera™ UC Prep (online SFE-SFC configuration)

Various collection modes (i.e., stacked-fraction, multi-fraction etc) are available, offering flexible solutions based on specific needs. The Nexera UC Prep enables a stepwise extraction process, combining static extraction (SE) and dynamic extraction (DE) (Fig. 2). During SE, the extraction fluid accumulates in the vessel, initiating the solubilization of the solid sample. DE further facilitates solubilization while transferring the dissolved compounds to the column. Solid-state injection minimizes peak distortion and precipitation by dissolving the sample directly into the extraction fluid, where the pressurized $CO_2$/co-solvent mixture exhibits lower polarity than pure solvents.

Although online SFE-SFC for solid-state injection is promising, its implementation can be challenging and requires a thorough understanding of parameter effects on sample loading.

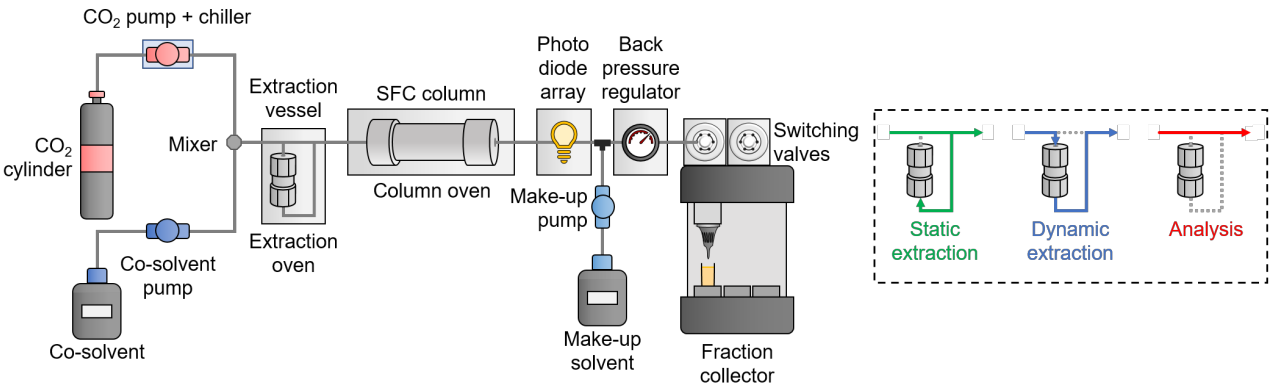


Fig. 2 Schematic representation of the semi-prep. on-line SFE-SFC system[1]

## ■ Model compounds

Semi-prep. online SFE-SFC was employed to purify ibuprofen from two structurally related compounds, 4'-isobutylacetophenone and valerophenone (Fig. 3).

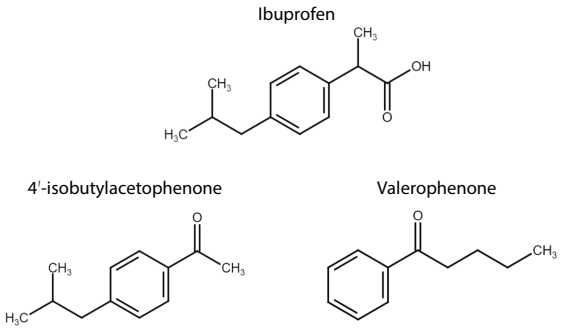


Fig. 3 Structures of model compounds

## ■ Ibuprofen solubility and offline SFC method

The solubility of ibuprofen in a pressurized $CO_2$/MeOH mixture were investigated. Optimal solubility was reached at 20% of MeOH, a value used to maximize sample loading during extraction[1)].

In parallel, an offline SFC method was optimized at the analytical scale, before scaling up to semi-prep. offline SFC (Table 1 and Fig. 4)[1)].

Table 1 Semi-prep. offline SFC-PDA conditions

| | |
|---|---|
| Column | : Shim-pack™ UC-PolyVP (250 mm x 20 mm I.D., 5 µm)*1 |
| Mobile Phase | : 30% of MeOH in $CO_2$ |
| Flowrate | : 60.0 mL/min |
| Column Temp. | : 25°C |
| BPR Pressure | : 10 MPa |
| BPR Temp. | : 50°C |
| Detection | : 220 nm (PDA with a high-pressure flow cell) |
| Injection Volume | : 50 µL in MeOH |

*1: P/N: 227-32511-11

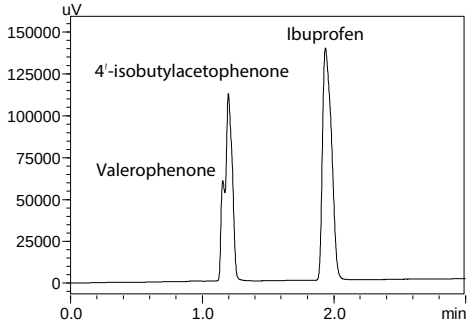


Fig. 4 Semi-prep. offline SFC-PDA chromatogram for the analysis of ibuprofen and structurally related compounds

Since the goal was to isolate ibuprofen from its structurally related compounds, the co-elution of valerophenone and 4'-isobutylacetophenone was not an issue. The Shim-pack UC PolyVP, featuring a poly(4-vinylpyridine) ligand, was selected for its selectivity and good retentivity for ibuprofen. Due to the direct transfer (without split) from the extraction vessel to the column, selecting a highly retentive column for the target compound was critical (Fig. 2). Indeed, a column with a poor retentivity will lead to band broadening due to a poor trapping efficiency[2)]. The method operates in isocratic conditions to simplify the subsequent semi-prep. online method. Using 30% of methanol allows to reduce the analysis time while maintaining a good separation of ibuprofen and structurally related compounds.

## ■ Effect of co-solvent ratio

The effect of co-solvent ratio during SE and DE was investigated. Five successive extraction cycles were performed using the same vessel. The ibuprofen peak area was measured for each cycle, and the values from all five cycles were summed for each experimental condition before comparison. PDA saturation at 220 nm was observed for the ibuprofen peak during the first cycle under all tested conditions. To address this, relative quantification was conducted at 240 nm, where PDA saturation was avoided. To evaluate the impact of co-solvent ratio on sample loading, 5% or 20% of MeOH were used during SE and DE (Table 2 and Fig. 5).

Table 2 Semi-prep. offline SFC-PDA conditions for co-solvent ratio investigation

| | |
|---|---|
| **[Extraction vessel]** | |
| Ext. vessel size | : 5 mL vessel (SUS type)*2 + 1 mL spacer*3 |
| Sample prep. | : 500 mg Ibuprofen<br>: 5 µL 4'-isobutylacetophenone<br>5 µL Valerophenone |
| **[SFE]** | |
| Ext. Duration | : *Static extraction:* 1 min<br>: *Dynamic extraction:* 1 min |
| Ext. Fluid | : *Static extraction:* x% of MeOH in $CO_2$<br>: *Dynamic extraction:* x% of MeOH in $CO_2$ |
| Flow rate | : 60 mL/min |
| Ext. Vessel Temp. | : Room temperature |
| BPR Pressure | : 10 MPa |
| BPR Temp. | : 50°C |
| **[SFC]** | |
| Column | : Shim-pack UC-PolyVP (250 mm x 20 mm I.D., 5 µm)*1 |
| Mobile Phase | : 30% of MeOH in $CO_2$ |
| Flow rate | : 60.0 mL/min |
| Column Temp. | : 25°C |
| BPR Pressure | : 10 MPa |
| BPR Temp. | : 50°C |
| Detection: | : 240 nm (PDA with a high-pressure flow cell) |

*1: P/N: 227-32511-11; *2: P/N: 228-58081-81; *3: P/N: 228-69904-41

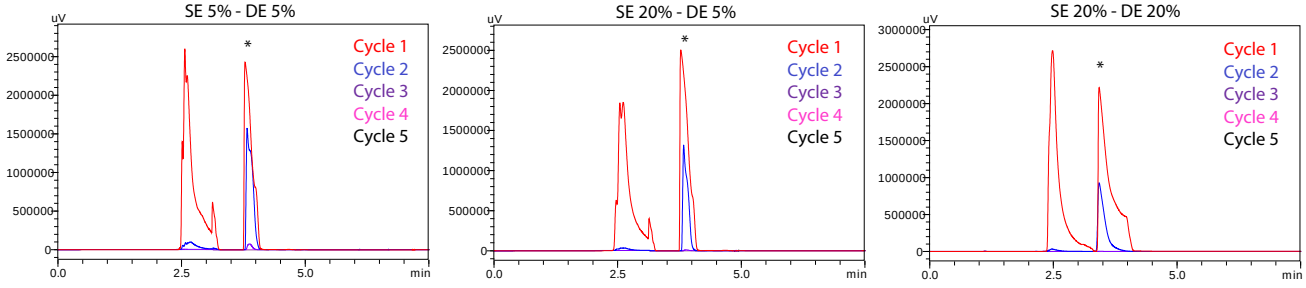


Fig. 5 Semi-prep. on-line SFE-SFC-PDA (240 nm) chromatograms for the analysis of ibuprofen and structurally related compounds after solid-state injection, depending of co-solvent ratio. Ibuprofen peak is highlighted by an asterisk.

Relative quantification results are summarized in Table 3.

Table 3 Ibuprofen proportion based on peak areas (240 nm) per semi-prep. online SFE-SFC-PDA cycle, depending on co-solvent ratio during extraction. All experiments were conducted with a SE of 1 min and a DE of 1 min.

| Cycle number | Ibuprofen proportion based on peak areas (%) | | |
|---|---|---|---|
| | SE 5% - DE 5% | SE 20% - DE 5% | SE 20% - DE 20% |
| Cycle 1 | 64.9% | 75.6% | 80.0% |
| Cycle 2 | 33.9% | 24.2% | 20.0% |
| Cycle 3 | 1.2% | 0.2% | 0.0% |
| Cycle 4 | 0.0% | 0.0% | 0.0% |
| Cycle 5 | 0.0% | 0.0% | 0.0% |

The increase of co-solvent ratio to reach optimal solubility (20%) increased the sample loading during the first cycles (Table 3). Although larger amounts were injected with "SE 20% - DE 20%", ibuprofen peak shape also had to be considered. Due to a better peak shape and close performances, "SE 20% - DE 5%" was used for further optimization (Table 3 and Fig. 5).

## ■ Impact of extraction duration

The effect of SE and DE duration was also investigated. For each condition, five cycles were performed using the same vessel. Experimental conditions and results are detailed in Table 4 and Fig. 6. Relative quantification results are summarized in Table 5.

Table 4 Semi-prep. offline SFC-PDA conditions for extraction duration investigation

| | |
|---|---|
| **[Extraction vessel]** | |
| Ext. vessel size | : 5 mL vessel[*2] + 1 mL insert[*3] |
| Sample prep. | : 500 mg Ibuprofen<br>: 5 µL 4′-isobutylacetophenone<br>5 µL Valerophenone |
| **[SFE]** | |
| Ext. Duration | : *Static extraction:* a min<br>: *Dynamic extraction:* b min |
| Ext. Fluid | : *Static extraction:* 20% of MeOH in $CO_2$<br>: *Dynamic extraction:* 5% of MeOH in $CO_2$ |
| Flow rate | : 60 mL/min |
| Ext. Vessel Temp. | : Room temperature |
| BPR Pressure | : 10 MPa |
| BPR Temp. | : 50°C |
| **[SFC]** | |
| Column | : Shim-pack UC-PolyVP<br>(250 mm x 20 mm I.D., 5 µm)[*1] |
| Mobile Phase | : 30% of MeOH in $CO_2$ |
| Flow rate | : 60.0 mL/min |
| Column Temp. | : 25°C |
| BPR Pressure | : 10 MPa |
| BPR Temp. | : 50°C |
| Detection | : 240 nm (PDA with a high-pressure flow cell) |

*1: P/N: 227-32511-11; *2: P/N: 228-58081-81; *3: P/N: 228-69904-41

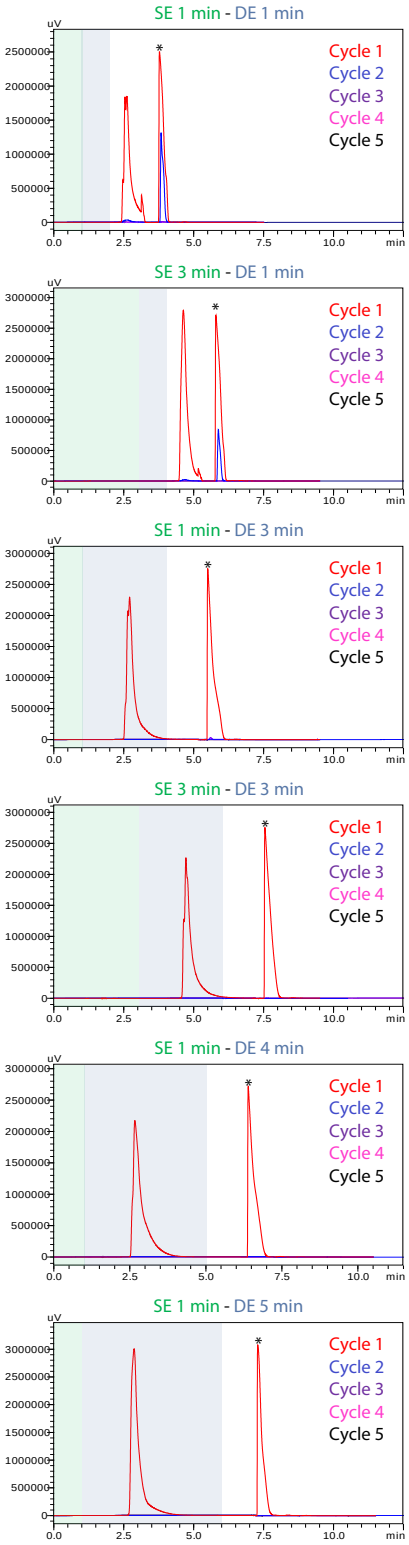


Fig. 6 Semi-prep. online SFE-SFC-PDA (240 nm) chromatograms for the analysis of ibuprofen and structurally related compounds after solid-state injection, depending on extraction duration. Ibuprofen peak is highlighted by an asterisk.

Table 5 Ibuprofen proportion based on peak areas (240 nm) per semi-prep. online SFE-SFC-PDA cycle, depending on extraction duration. All experiments were conducted with a SE of 1 min and a DE of 1 min.

| Cycle number | Ibuprofen proportion based on peak areas (%) | | | | | |
|---|---|---|---|---|---|---|
| | SE 1 min DE 1 min | SE 1 min DE 3 min | SE 3 min DE 1 min | SE 3 min DE 3 min | SE 1 min DE 4 min | SE 1 min DE 5 min |
| Cycle 1 | 75.6% | 85.9% | 99.6% | 99.9% | ~100.0% | ~100.0% |
| Cycle 2 | 24.2% | 14.1% | 0.4% | 0.1% | 0.0% | 0.0% |
| Cycle 3 | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Cycle 4 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Cycle 5 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

Increasing SE duration slightly enhanced sample loading during the first cycles, as observed when comparing "SE 1 min – DE 1 min" and "SE 3 min – DE 1 min". However, it was strongly impacted by a longer DE, as highlighted by the results of "SE 1 min – DE 3 min". Extending both SE and DE durations ("SE 3 min – DE 3 min"), allowed for nearly the entire sample to be injected in the first cycle. Considering those results, it may be possible to inject the entire sample within a single cycle simply by extending DE duration. Two additional conditions ("SE 1 min – DE 4 min" and "SE 1 min – DE 5 min") with an increase DE duration were tested. As expected, same tendency as before was observed along with the increase of DE duration. Further increasing DE to 4 or 5 minutes enabled the injection and purification of 500 mg of ibuprofen in a single cycle, achieving approximately 100% recovery.

## ■ Large-scale solid-state injection

The large amount injection had two objectives:

- Investigate if solid-state injection is suitable for few grams of sample and check the recovery.
- Confirm the need to increase co-solvent ratio when using a longer dynamic extraction.

For this experiment, the extraction vessel insert was removed to use the full 5 mL volume of the extraction vessel. As a result, amounts of ibuprofen and structurally related compounds were increased fivefold. The optimal conditions previously obtained were applied and are summarized in Table 6. However, two co-solvent ratios were tested during SE: 5% or 20% of MeOH in $CO_2$.

Table 6 Semi-prep. online SFE-SFC-PDA conditions for large-scale solid-state injection

| | |
|---|---|
| **[Extraction vessel]** | |
| Ext. vessel size: | 5 mL vessel*2 |
| Sample prep.: | 2500 mg Ibuprofen<br>25 µL 4′ -isobutylacetophenone<br>25 µL Valerophenone |
| **[SFE]** | |
| Ext. Duration: | *Static extraction:* 1 min<br>*Dynamic extraction:* 4 min |
| Ext. Fluid: | *Static extraction:* 5% or 20% of MeOH in $CO_2$<br>*Dynamic extraction:* 5% of MeOH in $CO_2$ |
| Flow rate: | 60 mL/min |
| Ext. Vessel Temp.: | Room temperature |
| BPR Pressure: | 10 MPa |
| BPR Temp.: | 50°C |
| **[SFC]** | |
| Column: | Shim-pack UC-PolyVP (250 mm x 20 mm I.D., 5 µm)*1 |
| Mobile Phase: | 30% of MeOH in $CO_2$ |
| Flow rate: | 60.0 mL/min |
| Column Temp.: | 25°C |
| BPR Pressure: | 10 MPa |
| BPR Temp.: | 50°C |
| Detection: | 240 nm (PDA with a high-pressure flow cell) |

*1: P/N: 227-32511-11; *2: P/N: 228-58081-81

Fractions were collected for each cycle, and the ibuprofen content was quantified by analytical SFC-PDA using external calibration (Fig. 7). The calibration curve (25 to 10000 mg/L, n=2) exhibited excellent linearity, with an $R^2 = 0.9998$. For "Cycle 1" and "Cycle 2", 125-fold and 12.5-fold dilutions with MeOH were performed prior SFC quantification. For fractions from "Cycle 3", "Cycle 4", and "Cycle 5", SFC quantification was conducted without dilution.

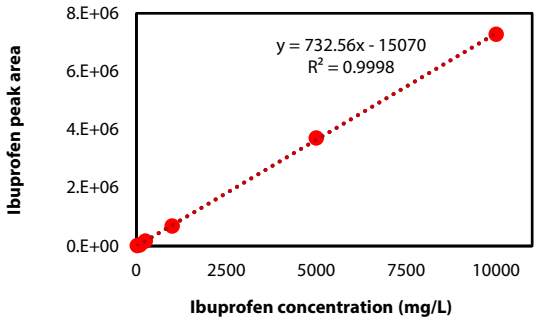


Fig. 7 Ibuprofen calibration curve (duplicate injection of calibration solutions in MeOH at 25, 100, 250, 1000, 5000 and 10000 µg/mL). Mobile phase: isocratic 30% of MeOH in $CO_2$; flow rate: 3 mL/min; column: Shim-pack UC PolyVP 250 mm x 4.6 mm I.D., 5 µm; oven temperature: 25°C; BPR: 10 MPa / 50°C; wavelength: 220 nm.

Quantified ibuprofen recovery weights and rates are reported in Table 7.

Table 7 Ibuprofen quantified (mg) and recovery rate per semi-prep. online SFE-SFC fraction, depending on co-solvent ratio used during static extraction.

| Cycle number | SE 5% - DE 5% | | SE 20% - DE 5% | |
|---|---|---|---|---|
| | Ibuprofen quantified (mg) | Recovery rate (%) | Ibuprofen quantified (mg) | Recovery rate (%) |
| Cycle 1 | 2257.2 | 89.7% | 2465.2 | 98.4% |
| Cycle 2 | 254.5 | 10.1% | 34.1 | 1.4% |
| Cycle 3 | 2.5 | 0.1% | 1.8 | 0.1% |
| Cycle 4 | 1.5 | 0.1% | 1.6 | 0.1% |
| Cycle 5 | 1.5 | 0.1% | 2.1 | 0.1% |
| Solid-state injection recovery rate (%) | 100.7% | | 100.2% | |

Recovery rates were very close to expected values, reaching 100.7% and 100.2% for "SE 5% - DE 5%" and "SE 20% - DE 5%," respectively. As previously observed, the co-solvent ratio used during SE played a crucial role in maximizing solid-state injection. Increasing the co-solvent ratio from 5% to 20% allowed for the injection of over 200 mg of additional ibuprofen during "Cycle 1" (2257.2 mg vs. 2465.2 mg). Moreover, using 20% co-solvent during SE enabled the injection and purification of nearly the entire 2.5 g of ibuprofen loaded into the vessel in a single cycle, achieving a recovery rate of 98.4%.

## ■ Conclusion

These results demonstrated the efficiency of solid-state injection for sample quantities ranging from hundreds to thousands of milligrams. However, for a successful application of the methodology, the following considerations should be addressed during method development:

- Candidates for solid-state injection: Any molecule suitable for extraction by SFE or analysis by SFC is a potential candidate for solid-state injection.
- Column selection: The selected column must provide sufficient retention for the target compound(s).
- Static extraction: The co-solvent ratio can be adjusted to match optimal solubility conditions of the target compound(s). A duration of 1 minute is sufficient.
- Dynamic extraction: A low co-solvent ratio should be preferred to prevent band broadening. A longer duration enables large sample loading and should be maximized.

Utilizing the extraction oven as “solid-state autosampler” allowed the progressive solubilization and rapid loading of the target compound, minimizing injection issues. Opting for solid-state injection instead of liquid injection can be advantageous for compounds that are difficult to solubilize or that degrade rapidly after solubilization. This approach opens the door to interesting applications such as racemate mixture purification, drugs deformulation etc.

<References>

1) Gros et al., J. Chromatogr. Open, 2025, Volume 7, 100206.

2) Gros et al., Trends Anal. Chem., 2021, 144, 116433.



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