A New Standard in Analytical Workflow Design

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Introduction

Current analytical methodology typically consists of a separate off-line sample preparation technique that is followed by a chromatographic analysis. Sample preparation and manual transfer to the analytical instrument often consumes a majority of the analyst’s time and effort. Recently, an innovative new concept was introduced that greatly reduces sample preparation times and the variability associated with manual procedures.

This new technique automates the sample preparation and analysis of samples by supercritical fluid extraction of compounds from the sample matrix, which are then transported to the analytical column for analysis without any human intervention. A number of applications that include food, environmental, and pharmaceutical areas will be shown that show the flexibility of this technique.

SFC Review

• Supercritical CO$_2$ is a fluid state of carbon dioxide where it is held at or above its critical temperature (31.1 °C) and critical pressure (1,070 psi)
• Rule-of-thumb: any molecule that dissolves in methanol (or less polar solvents) is compatible with SFC
• CO$_2$ at its critical point is non-polar, solvent strength is increased by using a polar co-solvent
• The benefits of supercritical fluids are still retained with the addition of co-solvents that greatly expand the application range. (subcritical fluid chromatography)
SFC Advantages vs. LC and GC

Lower viscosity of the mobile phase
- faster analysis (5 to 10 x faster)
- less pressure drop across the column
- better sensitivity

Greater separation efficiency than that of HPLC (chiral)
“Green” technique – reduced organic solvent usage/waste

Little residual solvent obtained in preparative chromatography
Analysis of non-volatile, polar or adsorptive solutes without derivatization
Analysis of thermally labile compounds
Preparative chromatography

System Configuration – SFE-SFC-MS/MS

Online SFE – SFC – MS System
Splitless BPR Improves Sensitivity

- High sensitivity detection
- Improved sensitivity due to low dead volume BPR
- Splitless injection into the MS without band broadening

Streamlined, On-line Sample Prep

- SFE (supercritical fluid extraction) sample preparation

Up to 48 samples can be extracted and analyzed in an automated workflow using the Rack Changer.

approx. 30 min time saving / sample

Patented absorbent effective for dehydration of samples with high water content
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- Conventional sample preparation method (QuEChERS)

Preserves Labile Compounds

Analysis of coenzyme Q_{10} in supplements
- Conventional Method: Coenzyme Q_{10} is prone to oxidation

Nexera UC: No oxidation with SFE

Extraction with 5 % MeOH in CO₂ for 4 min

Gradient: 5 %B (0 min – 4 min for SFE)
5 % - 50 %B in 5 min

![Diagram]
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## DBS Analysis

### Analysis of biomarkers from dried blood spot (DBS)

- **Conventional Method:** time consuming

  ![Conventional Method Diagram]

- **Nexera UC:**

  ![Nexera UC Diagram]

## Polymer Additives Analysis

### Extraction of polymer additives

- **Conventional Method:** 10 – 20 h soxlet extraction
- **Nexera UC:** 7 min CO₂ extraction
  - Crushed polymer samples are placed in the extraction vessel
  - After 7 min CO₂ extraction, the sample is ready for SFC analysis

![Polymer Additives Analysis Graphs]
Cleaning Analyses

Cleaning validation

- Conventional Method:
  - Sampling – Solvent extraction – Concentration – HPLC analysis
  - Organic solvents can’t be used for TOC analysis
- Nexera UC:
  - Swab is enclosed in the extraction vessel for SFE

Analysis of API in a Tablet

Approx. 2mg of crushed tablet sample was weighed and transferred to the vessel for analysis. Recovery was calculated by comparing to the standard that is equivalent to the amount of API in 2mg of the tablet.
Summary

- A new automated online sample preparation/chromatography system that uses supercritical fluids was recently introduced.
- The system can greatly reduce sample preparation times when compared to conventional methods like QuEChERS.
- Automated supercritical fluid extraction (SFE) can reduce solvent waste while improving reproducibility of results.
- SFE was shown to be a viable technique for a number of applications.