ISOLATION OF FLAVOR COMPOUNDS FROM NATURAL PRODUCTS USING AN SFE-SFC WORKFLOW

Jacquelyn Runco
Waters Corporation, Pittsburgh, PA, USA

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INTRODUCTION

Naturally occurring target compounds are used in a variety of consumer products from pharmaceuticals and nutraceuticals to flavors and fragrances. Currently, natural flavors are in high demand, a trend that reflects increased consumer awareness and preference for traditional or organic food. [1]

In a supercritical fluid workflow, CO_2 with or without the addition of an organic modifier, is used to extract (SFE) and purify (SFC) target compounds. The CO_2 used as a solvent is safe and the extracts produced by this process are free from biological contaminants, have longer shelf life, high potency, and address major international concerns regarding residual solvent concentration.[2] In this study, a complete SFE-SFC workflow will be demonstrated using the MV-10 ASFE system (MV-10) for extraction and the Prep 80a SFC System (SFC 80) for purification of two compounds; vanillin from whole vanilla beans and cinnamic acid from organic ground cinnamon. This workflow includes SFE method development, analytical method development, scale-up, purification, and fraction analysis. The process can be adapted to purify target compounds from a variety of natural products and matrices.

METHODS

Sample Description

- Three 2g samples of Mexican vanilla beans (diced)
- Three 2g samples of organic ground cinnamon

Extraction
System: MV-10 ASFE System
Vessel Volume: 5 mL
Software: ChromScope
Mobile Phase A: CO_2
Mobile Phase B: Ethanol (200 Proof HPLC Grade)
Flow Rate: 10mL/min
Composition: see Table 1
Make-up Solvent: Ethanol (200 Proof HPLC Grade)
Make-up flow: see Table 1
Pressure: 300 Bar
Temperature: 40°C
Method Steps:
Dynamic 1: 3 min
Static: 60 min
Dynamic 2: 30 min

Vessel #	Mobile Phase B (%)	Mobile Phase B (mL/min)	Make-up flow (mL/min)
1	0	0	1.5
2	5	0.5	1
3	10	1	0.5

***Table 1**: MV-10 SFE method screening conditions*

Preparative SFC
System: Prep 80q SFC System with a 2489 UV/Vis detector
Software: ChromScope
Mobile Phase A: CO_2
Mobile Phase B: Ethanol (Reagent HPLC Grade)
Flow Rate: 72 mL/min
Gradient: see figures
Pressure: 220 Bar
Temperature: 40°C
UV: 267nm
Preparative Column: Viridis 2-EP Column (19x150mm, 5µm)
Injection Volume: 2 mL

Fraction Analysis
System: ACQUITY UPC^2 System with an ACQUITY UPC^2 PDA detector
Software: MassLynx
Mobile Phase A: CO_2
Mobile Phase B: Ethanol (200 Proof HPLC Grade)
Flow Rate: 1.5 mL/min
Gradient: see figures
Pressure: 150 Bar
Temperature: 40°C
PDA: Scan: 220-400nm
Absorbance Compensated: 267nm
Reference: 320-400nm
Analytical Column: ACQUITY UPC$^{2\ TM}$ BEH 2-EP Column (3x100mm, 1.7µm)
Injection Volume: 2 µL

RESULTS & DISCUSSION

In SFE method development, extracts obtained at each set of conditions are evaluated for target compound yield and purity. Overall, yield is important, but lower impurity levels in the extract results in easier SFC method development and purification. In food related applications such as the ones presented here, CO_2-only conditions are preferred because of improved consumer safety and the elimination of organic solvent waste.

SFE method development was performed on the MV-10 ASFE System, where software controlled automation allows the user to program various extraction conditions for multiple samples and run them unattended. Extractions were performed at 0%, 5%, and 10% ethanol on three 2g samples for both the vanilla and the cinnamon. The amounts of vanillin in the vanilla extract and cinnamic acid in the cinnamon extract are recorded in Table 2. Extract analysis and quantitation was performed using calibration curves (R^2=1.0000) on the UPC^2 system.

In the case of the vanilla extracts, there was little statistical difference in the extraction yields, so the higher purity 0% (CO_2-only) extract was selected for purification. In contrast, the cinnamon extracts showed a significant yield difference, with the 10% method yielding four times more than when no ethanol was used, but with little difference in impurity levels. Chromatograms of the extracts are displayed in Figure 1.

Sample	Target Compound	Yield (mg)		
		0% ethanol	5% ethanol	10% ethanol
Vanilla	Vanillin	24.9	24.7	22.9
Cinnamon	Cinnamic Acid	0.45	1.38	1.85

***Table 2**: Extraction yields obtained at 0%, 5%, and 10% ethanol extraction conditions. Each of the 6 extractions was performed on 2g of starting material*

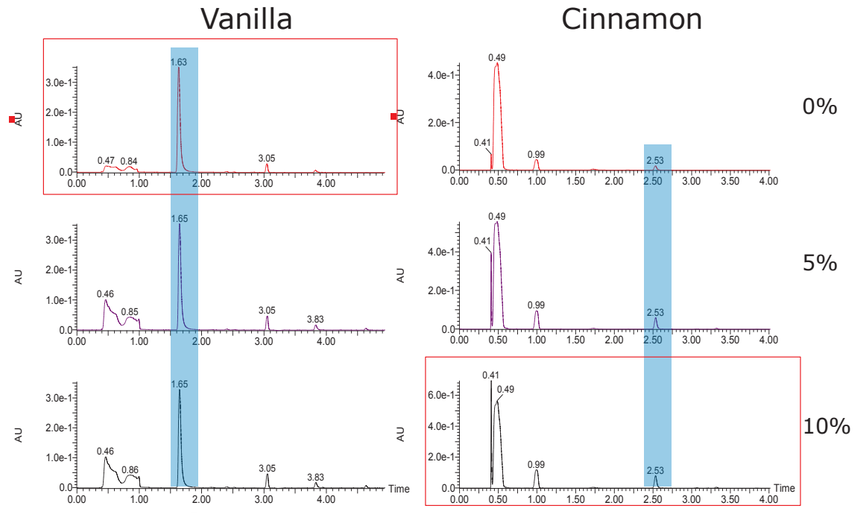


***Figure 1:** UPC^2 separations of the vanilla bean and cinnamon extracts obtained using 0%, 5% and 10% ethanol. 1.5 mL/min, 2 – 20% gradient in 5 min, 150 Bar, 40°C, 2 µL Injection, PDA Absorbance Compensated at 267 nm. The target peaks are highlighted in blue.*

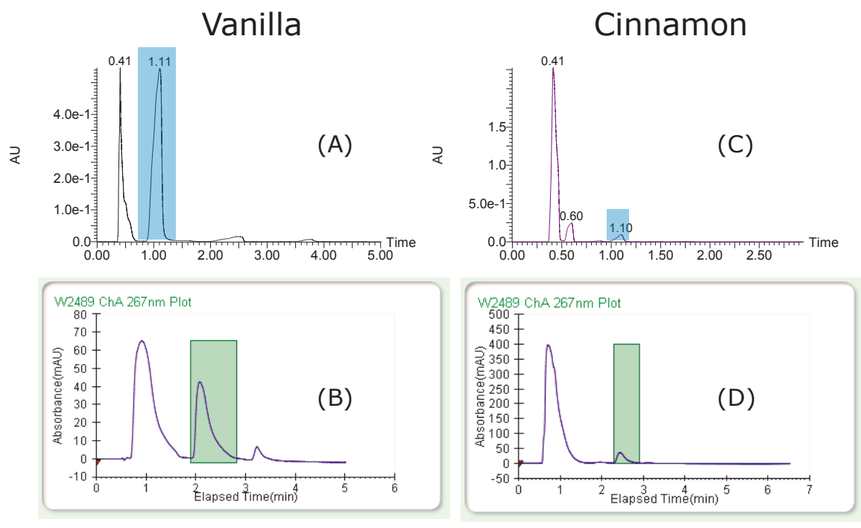
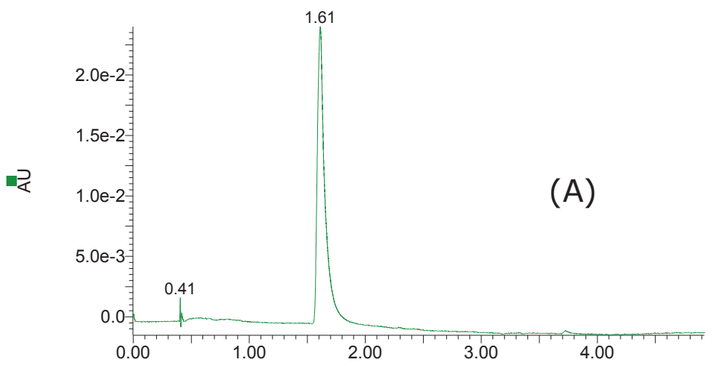


***Figure 2**: Chromatograms of the vanilla and cinnamon extracts using the modified gradients on the UPC^2 (A) and (C) and the scaled-up separations on the SFC 80 with green collection marks (B) and (D). Gradient and flow conditions are (A) 1.5 mL/min, 5-15% B in 3 min, (B) 72 mL/min, 5-15% B in 4.5 min, (C) 1.5 mL/min, 8-20% B in 3 min, and (D) 72 mL/min, 8-20% B in 5 min. Injection volumes were 10 µL for (A) and (C), 2 mL for (B) and (D).*

Generally when purification is the goal, it is more practical for method development to be performed at the analytical scale to save solvent, time and sample. The initial gradients on the UPC^2 were 2-20% ethanol in 5 minutes (1.44%/cv). Based on their retention times, the vanillin eluted around 6% while the cinnamic acid eluted around 9%. In order to optimize the separation for purification, the gradients were modified to 5-15% ethanol in 3min for the vanilla extract and 8-20% ethanol in 3 mins for the cinnamon extract. Under these conditions, separation was maintained up to 10µL for both samples while reducing the overall run time (Figure 2 (A) and (C)).

The modified UPC^2 method parameters were scaled for the 19x150mm Viridis 2-EP prep column, resulting in a 5-15% 4.5 min gradient for the vanillin purification and a 8-20% 5 min gradient for the cinnamic acid purification. Due to the small particle size of the column and tubing ID on the UPC^2 system, the system pressure was 260 bar (110 bar pressure drop across the system). The two systems needed to be operated at similar pressures to keep consistent CO_2 density, which ensures consistent separation on scale-up. To maintain a 260 bar system pressure on the SFC 80, the BPR pressure was set to 220 bar (40 bar pressure drop). The calculated geometric scale-up was 600µL, but the experimental separation allowed for much higher loading, so 2 mL injections were used (Figure 2 (B) and (D)).

The SFC 80 purification system is designed for bulk purification, making it useful for applications where large amounts of a single sample need to be purified. While various collection methods can be used on the SFC 80, in this case fractions were collected by time. The fractions collected on the SFC 80 were analyzed on the UPC^2 (Figure 3). Purity and recovery for both samples is displayed in table 3. For both samples the recovery was greater than 90%, and purity was significantly improved compared to the raw extracts.



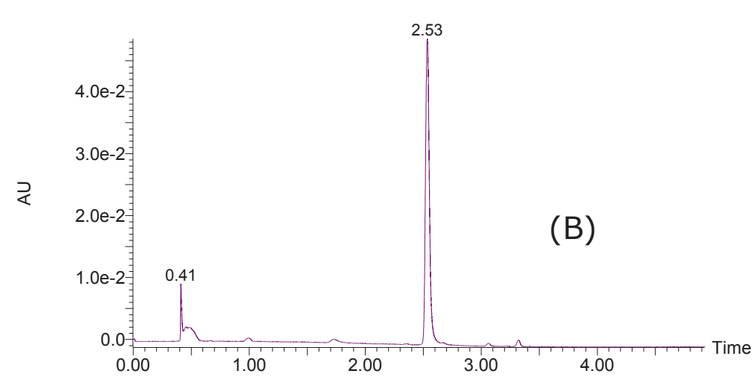


***Figure 3**: UPC^2 analysis of the (A) vanillin and (B) cinnamic acid fractions collected on the SFC 80. 1.5 mL/min, 2 – 20% gradient over 5 min, 150 Bar, 40°C, 2 µL Injection, PDA Absorbance Compensated at 267 nm.*

Sample	Target Compound	Purity	Recovery
Vanilla	Vanillin	100%	91%
Cinnamon	Cinnamic Acid	97%	93%

***Table 3**: Fraction purity and collection recovery achieved using the SFC 80.*

CONCLUSION

- **A total supercritical fluid workflow solution is presented for the extraction, purification and analysis of selected flavor compounds, utilizing less solvent and processing time than other traditional methods**
- **The MV-10 ASFE system allowed for simple method development by screening three solvent conditions using software controlled automated processes.**
- **Successful scale-up of the UPC^2 separation to the SFC 80 was demonstrated, taking advantage of fast method development on the UPC^2.**
- **Vanillin and cinnamic acid were successfully isolated from the raw extracts using the SFC 80 purification system.**
- **The entire workflow employed non-toxic CO_2 and ethanol as the mobile phase or extraction solvent which is ideal for food related applications by improving safety for consumers, and eliminating organic solvent waste**
- **The process presented can be adapted to isolate target compounds in many natural product applications.**

References

1. A.M. Rouhi, "Indulging the chemical senses", *C&EN*, July 14, 2003, 53-60

2. http://www.celkau.in/Crops/Spices/Vanilla/Vanilla_composition_and_vanillin_content.aspx

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