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Note d'application

# Enantiomeric and diastereomeric separations of fragrance and essential oil components using the ACQUITY UPC$^2$ System with ACQUITY UPC$^2$ Trefoil Columns

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## Abstract

In this application note, we have demonstrated the successful chiral separations of fragrance compounds on ACQUITY UPC$^2$ Trefoil AMY1 and CEL1 Columns using an ACQUITY UPC$^2$ System. The low system volume and extra-column volume of the UPC$^2$, combined with the reduced particle size of the ACQUITY UPC$^2$ Trefoil AMY1 and CEL1 Columns, enable superior, faster, and more efficient separations compared with traditional SFC and GC.

### Benefits

- Shorter analysis times compared to chiral GC.
- The 2.5-µm particle chiral stationary phases provide high efficiency enantiomeric separations for fragrance compounds.
- The low system volume and extra-column volume of the ACQUITY UPC$^2$ System enables superior, faster, and more efficient enantiomeric separations of fragrance compounds compared to traditional SFC.
- UPC$^2$ solvents are more compatible with mass spectrometry, compared to those used in normal-phase chiral HPLC, enabling superior real time peak identification.


## Introduction

Perception of aroma occurs at the olfactory membrane. This membrane is comprised in part of proteins and carbohydrates, which are chiral in nature. This makes it possible for the olfactory receptors to distinguish between enantiomers. Many enantiomers of fragrance molecules are perceived differently by our sense of smell.[1] For example, carvone is a chiral terpenoid where the R enantiomer smells like spearmint while the S enantiomer has the distinct odor of caraway seed.[2]

Chiral composition of fragrance molecules is important for many industries, including food, cosmetics, and consumer products, in controlling the olfactory perception of products.[1] In addition, chiral analyses are routinely performed to authenticate the natural sources of essential oils. Since naturally chiral sources of essential oils are generally more costly and have a greater perceived health benefit than their synthetic counterparts, rapid chiral analysis allows manufacturers to quickly exclude adulterated products containing inexpensive racemic synthetic materials at the time of purchase.[3]

Historically, chiral separations of fragrance compounds have primarily been carried out using chiral stationary phases (CSPs) in capillary gas chromatography (GC).[2,3,4] The analysis time often ranges from 15 to 50 minutes.[3] More recently, supercritical fluid chromatography (SFC) with CSPs has been applied to these separations, often resulting in comparable resolution and reduced run time.[5,6] Despite the great success in chiral separation by SFC, the associated instrumentation and CSPs have been slow to tap into the technology advancements that have taken place in the HPLC field. For example, one of most significant breakthroughs in HPLC in the past decade is the advent of Waters UPLC Technology, which utilizes sub-2-µm particles. ACQUITY UPLC Systems retain the practicality and principles of HPLC while increasing the overall interlaced attributes of speed, sensitivity, and resolution. Until very recently, the standard particle size for commercially available CSPs has remained 5 µm.

Convergence chromatography is the next evolution in SFC. The Waters ACQUITY UPC$^2$ System is a holistically designed system that has similar selectivity to normal-phase chromatography and is built upon proven UPLC technology.

UltraPerformance Convergence Chromatography (UPC$^2$) offers minimized system and dwell volume, enabling users to leverage the superior separation power inherent to smaller particle sizes.

We present herein the enantiomeric and diastereomeric separations of four fragrance compounds using Waters ACQUITY UPC$^2$ Trefoil AMY1 and CEL1 Columns on an ACQUITY UPC$^2$ System. Compared to the traditional method of analysis by GC, UPC$^2$ offered similarly high resolution with significantly shorter run times, resulting in improved productivity.

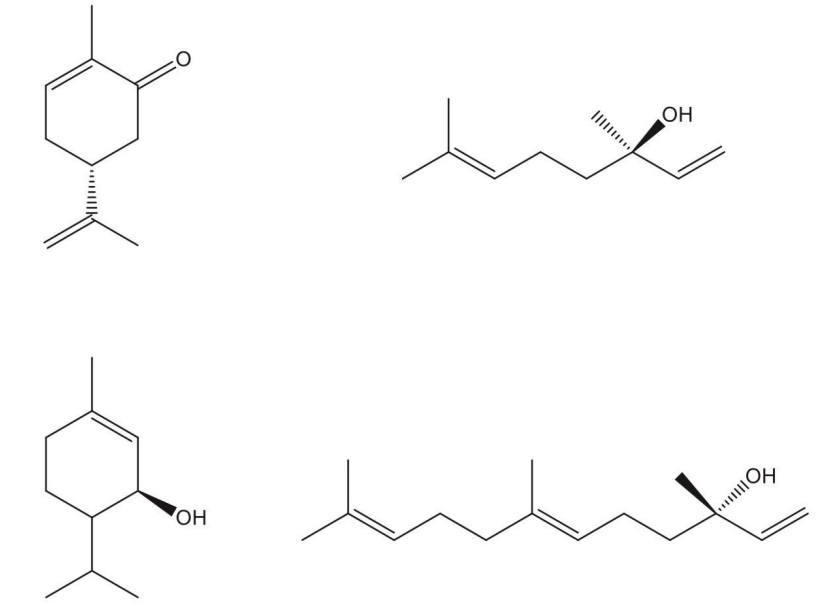


*Figure 1. Structures of the four fragrance compounds presented in this study.*

# Experimental

## Instrumentation

All experiments were performed on an ACQUITY UPC$^2$ System equipped with an ACQUITY UPC$^2$ PDA Detector and an ACQUITY TQ Detector. The system is controlled by MassLynx Software.

## Samples

The standard samples used in this study were purchased from TCI Americas, with their structures shown in Figure 1. Essential oils were purchased from various commercial sources. All samples were dissolved in tert-butyl methyl ether (TBME) for the analyses.

UPC$^2$ conditions

| | |
|---|---|
| Column: | ACQUITY UPC$^2$ Trefoil AMY1 or CEL1 (2.5 µm, 3.0 x 150 mm) |

| $UPC^2$ conditions | |
|---|---|
| Backpressure: | 1740 psi |
| Temperature: | 40 °C |
| Mobile phase A: | $CO_2$ |
| Mobile phase B: | Isopropanol. |
| MS: | APCI positive mode. |
| Other key parameters are listed in their respective figure captions. | |

## Results and Discussion

Figure 2 shows the $UPC^2$-UV chromatogram of carvone enantiomers on an ACQUITY $UPC^2$ Trefoil CEL1 Column. The enantiomeric pair was baseline resolved in less than 2.5 min (Figure 2C). The peak widths at half-height are 2-3 s. It is also interesting to note that there are detectable antipodes present in both single enantiomer standards (Figures 2A and 2B). In both cases, the minor peaks account for approximately 1% of the main peaks, resulting in a 98% enantiomeric excess (e. e.). This example clearly demonstrates a high efficiency chiral separation enabled by a 2.5-µm CSP on an ACQUITY $UPC^2$ System, resulting in short analysis time, sharp peaks, and improved detection sensitivity.

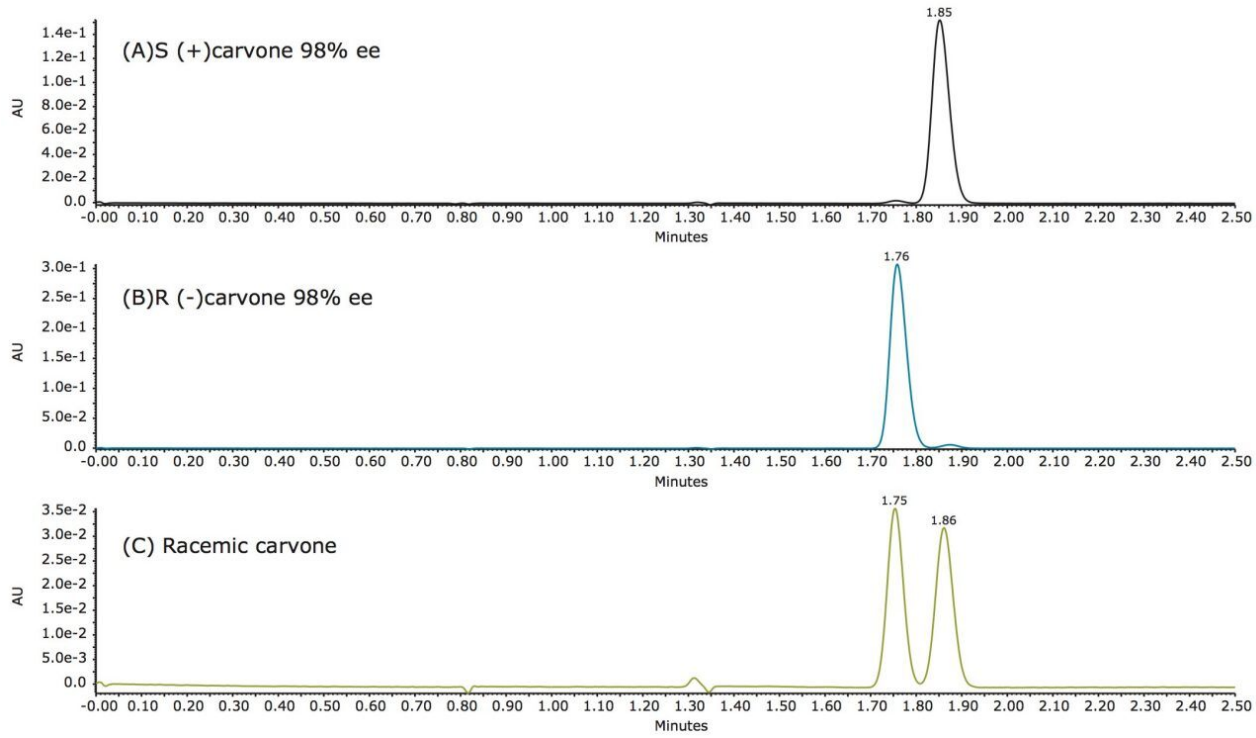


*Figure 2. UPC$^2$-UV chromatograms of the enantiomeric separation of carvone on an ACQUITY UPC$^2$ Trefoil CEL1 Column: (A) S (+) carvone; (B) R (-) carvone; and (C) racemic carvone. An isocratic method with 4% isopropanol was used. The flow rate was 0.9 mL/min.*

Linalool is a terpene alcohol with a soft floral odor, and can be found in different plant extracts. Figure 3A shows the enantiomeric resolution of the linalool standard on an ACQUITY UPC$^2$ Trefoil AMY1 Column. It is noted that the linalool standard was non-racemic (Figure 3A), suggesting the standard was derived from a natural source. The e. e. was estimated to be 40% in favor of the late eluting isomer. Figure 3B is the UPC$^2$-UV chromatogram of a commercially available lavender essential oil obtained under the same condition. The two linalool enantiomers were identified by both retention time and corresponding mass spectra (results not shown). It is noted that MS plays a critical role for the positive identification of the target analytes in a complex matrix. While bearing a similar selectivity to normal-phase LC, UPC$^2$ is inherently advantageous in incorporating MS detection due to its MS-friendly mobile phase. The linalool in this lavender essential oil exhibited a 92% e. e. in favor of the later eluting peak at 2.07 min.

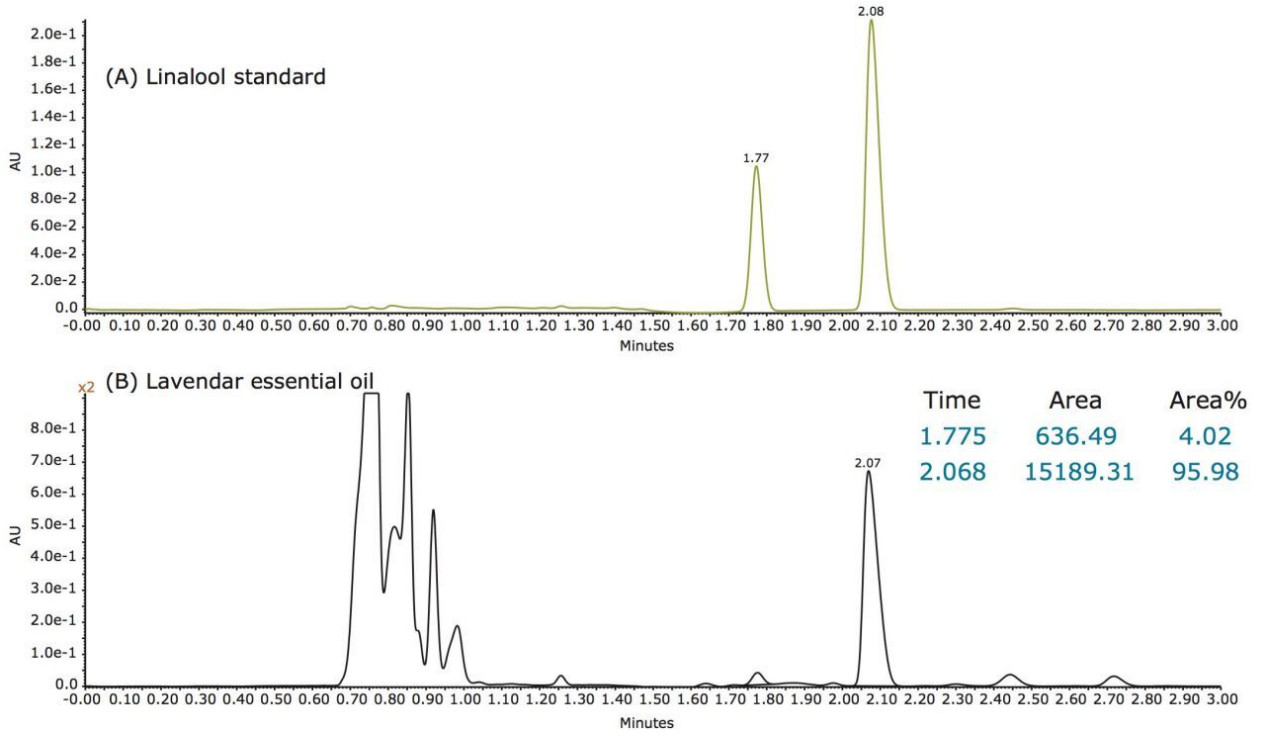


*Figure 3. UPC$^2$-UV chromatograms of (A) linalool standard (B) lavender essential oil on an ACQUITY UPC$^2$ Trefoil AMY1 Column. An isocratic method with 3% isopropanol was used for linalool. The flow rate was 1.0 mL/min.*

Similarly, terpinen-4-ol is a terpene with a pleasant conifer odor, and is a major constituent of tea tree oil. Figure 4A shows the enantiomeric resolution of the two isomers of a terpinen-4-ol standard on an ACQUITY UPC$^2$ Trefoil AMY1 Column. The terpinen-4-ol standard was nearly racemic (Figure 4A), suggesting its synthetic origin. Examination of a tea tree essential oil (Figure 4B) revealed that the terpinen-4-ol exhibited a 37% e. e. in favor of the early eluting isomer at 1.95 min.

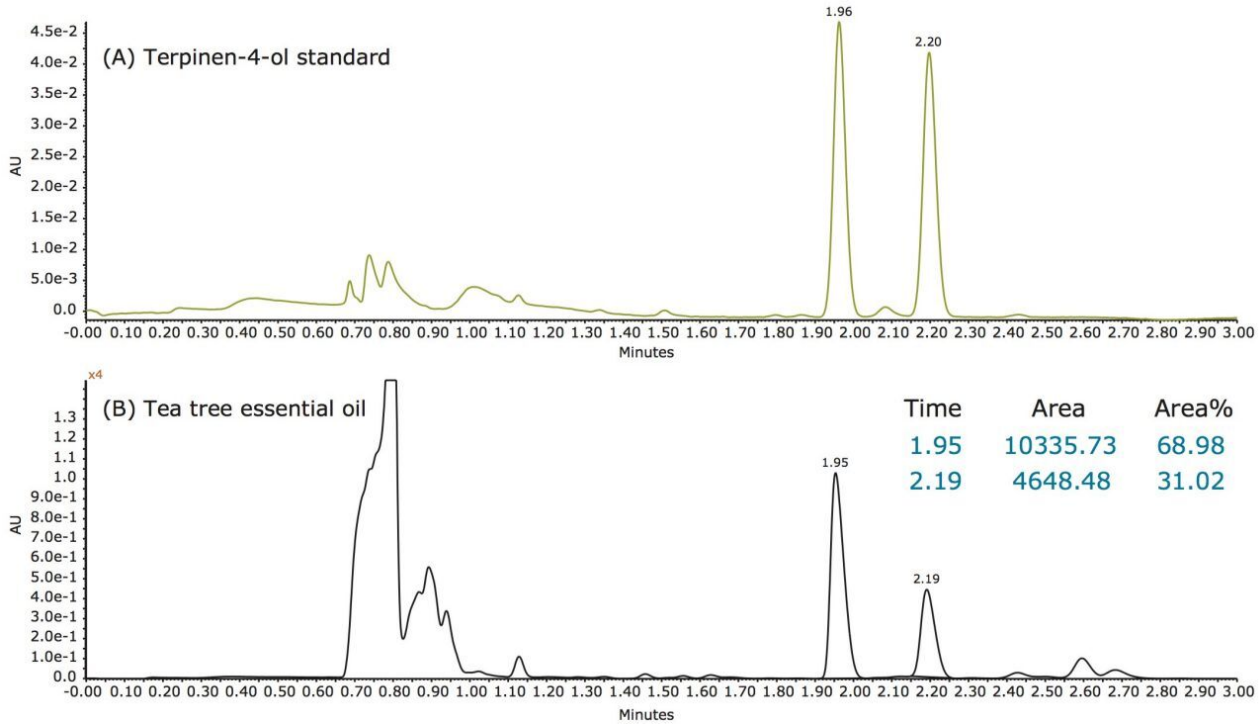


*Figure 4. UPC$^2$-UV chromatograms of (A) Terpinen-4-ol standard and (B) Tea Tree essential oil on an ACQUITY UPC$^2$ Trefoil AMY1 column. An isocratic method with 5% isopropanol was used. The flow rate was 1.0 mL/min.*

Nerolidol, which can be found in the neroli essential oil derived from the bitter orange plant, is a sesquiterpene with a pleasant woody odor reminiscent of fresh bark. The nerolidol molecule (Figure 1) contains a chiral center and a double bond generating cis/trans isomerism, resulting in four possible stereoisomers in a mixture. Figure 5 shows the simultaneous separation of all four nerolidol isomers on an ACQUITY UPC$^2$ Trefoil AMY1 column in less than 3 min. Figure 5B is the selected ion recording (SIR) for the isomeric mixture at *m/z* 205.2, corresponding to the $[(M+H)-H_2O]^+$ of nerolidol. The observation of the base peak of nerolidol resulting from the loss of water is consistent with other reports.[7]

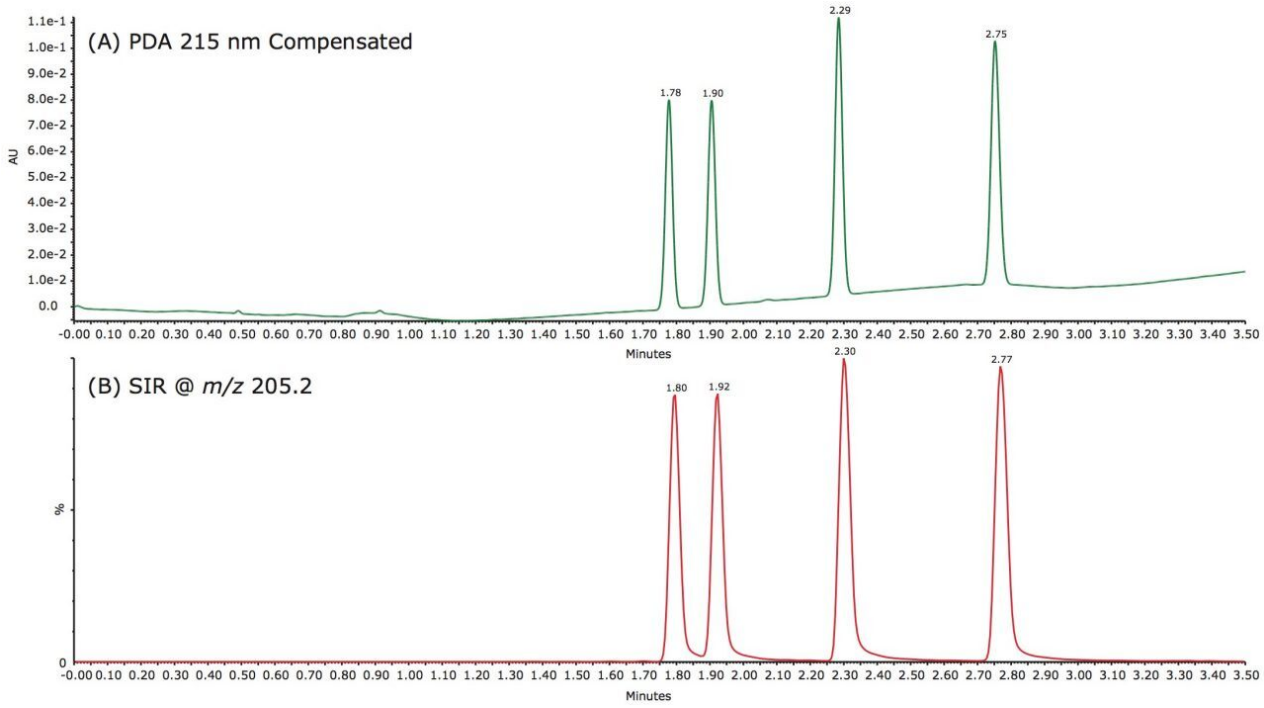


*Figure 5. UPC$^2$ chromatograms of a nerolidol standard separated on an ACQUITY UPC$^2$ Trefoil AMY1 Column: (A) UV at 215 nm with a compensation wavelength of 260-310 nm; and (B) SIR at m/z 205.2. The flow rate was 1.5 mL/min. A gradient of 2-7% isopropanol in 3.5 min was used.*

## Conclusion

In this application note, we have demonstrated the successful chiral separations of fragrance compounds on ACQUITY UPC$^2$ Trefoil AMY1 and CEL1 Columns using an ACQUITY UPC$^2$ System. The low system volume and extra-column volume of the UPC$^2$, combined with the reduced particle size of the ACQUITY UPC$^2$ Trefoil AMY1 and CEL1 Columns, enable superior, faster, and more efficient separations compared with traditional SFC and GC. The demonstrated analysis times range from 2 to 3 minutes, significantly shorter than the 15-50 minute analysis time typical for chiral GC,[3] allows for a fast authentication of the natural sources of essential oils. In all cases, the closely eluting isomers were baseline resolved. For the essential oil analysis, the oil samples were diluted and directly injected onto an ACQUITY UPC$^2$ System without tedious sample preparation. Furthermore, the inherent compatibility between UPC$^2$ and MS offered an unambiguous identification of the target analytes in a complex sample matrix. The high efficiency, short analysis time, and simple sample workup demonstrated in this study should be welcomed by industries where chiral analyses of

fragrance compounds are routinely performed.

## References


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