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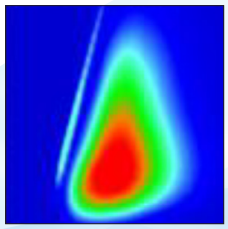
Fluorescence

A-TEEM™ Fluorescence Spectroscopy for the Quick Detection of Adulteration in Lavender Essential Oils



Application Note
Life Sciences
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Introduction

Aromatherapy is the practice of using essential oils for therapeutic benefit, an approach that has been utilized for centuries. Essential oils (EOs) are a complex amalgam containing a variety of naturally derived compounds that have been purported to improve one's physical and physiological well-being. Although most often used topically, some EOs are marketed to be inhaled or ingested, uses that demand exceptionally high-quality oils. That said, there is no centralized U.S. regulatory agency responsible to certify EOs for quality and purity. As the extraction of EOs from natural products often requires large quantities of the starting material to obtain appreciable yields, pure EOs can be quite expensive. Unethical manufacturers have an economic incentive to adulterate pure oils with cheaper alternatives that are difficult to detect by the standard analytical tools.

Adulteration of EOs can be achieved in several ways, as naturally derived compounds can be replaced by: 1) synthetic compounds; 2) different and less expensive plant strains; or 3) vegetable and mineral oils. The expense and analysis time to detect the different adulterant pathways can be prohibitive, and with no regulations, the extent to which EOs are analyzed varies among manufacturers, with little consistency. Consumers looking for low-cost options may not be buying the quality product that they think they are. Figure 1 shows the common components of lavender essential oils.

The American Botanical Council (ABC), American Herbal Pharmacopeia (AHP), and the Univ. of Mississippi's National Center for Natural Product Research (NCNPR) are working to address these issues as part of the Botanical Adulterants Prevention Program. They recently published a Laboratory Guidance Document (LGD) on English Lavendar Essential Oil (Shulha, 2023).

There are a variety of chromatographic and spectroscopic techniques recommended by the LGD, each with pros and cons. Separations methods (HPTLC, HPLC, HPLC-UV-MS) require reference standards, so the per-measurement cost can be quite high. GC-MS is the "gold standard" of EO analysis, but the approach cannot directly detect adulteration by vegetable oils and other non-volatile components. Standard spectroscopic approaches (NIR, FTIR, Raman) have lower sensitivity than separations, but tend to be very rapid measurement times, and the per-measurement cost is quite low. There is a growing interest and need for the development of a rapid and sensitive methodology that can be used to screen samples and assess the purity of lavender essential oils in a timely and cost-effective manner.

Here we present A-TEEM spectroscopy, a novel, robust, and extremely sensitive analytical technique comprising the best attributes of chromatography (sensitivity, selectivity, low limits of detection and quantification) and molecular spectroscopy (speed, low per measurement cost, and ability to operate in a manufacturing environment). The A-TEEM technique combines absorbance and fluorescence spectroscopy in a simultaneous measurement, implemented with a two-dimensional CCD detector for results in ~60 seconds, an order of magnitude faster than the typical point-by-point scanning approach. The result is an A-TEEM contour comprised of: 1) UV/Vis absorbance spectrum; 2) fluorescence EEM profile providing full fluorescence excitation and fluorescence emission spectra; and 3) automated correction of the Inner Filter Effect (IFE). Unlike traditional fluorescence EEMs that are uncorrected for IFE, the A-TEEM contour provides a true molecular fingerprint, enabling quantitative analysis of responsive components to ppb levels.

Experimental Procedure

A-TEEM Fluorescence Spectroscopy

Lavender essential oil was purchased from two different manufacturers, one that tests product quality with a third-party lab, and a second without that assurance. Both products are marketed as "Pure" Lavender Essential Oil from English Lavender Lavendula Angustifolia. A-TEEM measurements were acquired using an Aqualog® UV-800 spectrophotometer (Figure 2), which simultaneously collected absorbance-transmission (A-T) and fluorescence excitation – emission (A-TEEM) data. The excitation wavelength was set to a range of 240–800 nm with an increment of 5 nm, and the emission wavelength was set to a range of 246–823 nm with an increment of 4.66 nm. Measurements were performed under medium gain and with an integration time of 2.0 sec per single excitation point, with an overall measurement time of ~4 minutes.

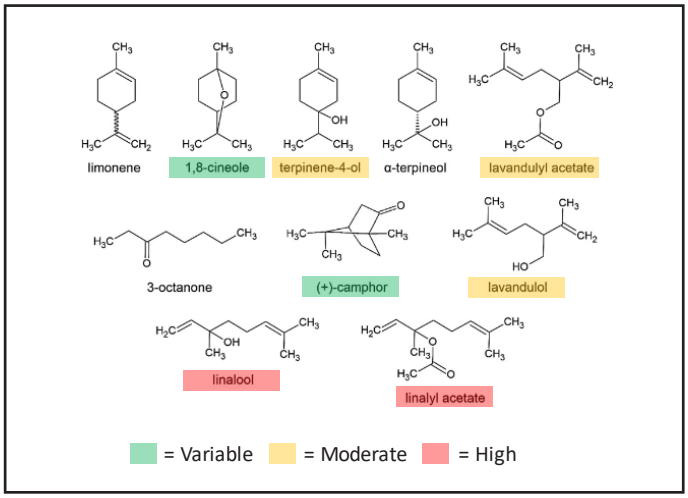

Figure 1: Prevalent components of lavender essential oil.

Samples were analyzed in quartz cuvettes with a pathlength of 1.0 cm and the cell temperature was temperature controlled at 20°C. Lavender essential oil samples were diluted in ethanol to facilitate a linear concentration relationship according to Beer-Lambert for the absorbance and fluorescence signal. Data acquisition and analysis was performed using Aqualog software (V4.3) and featured automatic spectral preprocessing including interpolation, correction of inner filter effects (IFE), Rayleigh masking (RM), and normalization (NRM) to the water raman peak area using an integration time of 2 seconds. Both corrected A-TEEM contours were normalized to 1. The A-TEEM of the EO sample tested for quality by the seller was subtracted from the A-TEEM of the other sample. The scale bar of the resultant A-TEEM was divided by 0.01 to reflect percent difference between samples.

Results & Discussion

Lavender essential oil is extremely rich in UV active components, requiring significant dilution of the samples to stay within the limits of Beer-Lambert linearity. Interestingly, the two brands of lavender essential oil require very different dilution factors to fall within this range: the lavender oil tested for quality by the manufacturer is diluted by a factor of 12,500, whereas the untested brand requires only 3000x dilution. This is the first indication of potential adulteration. The fluorescence A-TEEM profiles of the two samples are quite different as well, particularly in the deep UV region of the A-TEEMs (Figure 3).

Figure 2: Aqualog® benchtop spectrophotometer equipped with an autosampler.

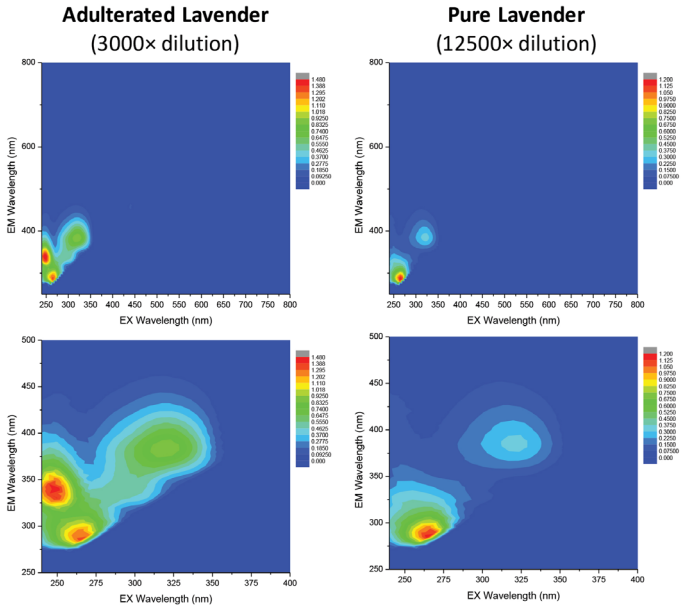

Figure 3: Fluorescence A-TEEM profiles of adulterated lavender essential oil (top left) and pure lavender essential oil (top right). Shown at the bottom is the zoomed regions of the fluorescing compounds of each sample.

A potential workflow for QC testing of lavender samples is to establish the A-TEEM molecular fingerprint of a “golden batch” against which “unknowns” are tested. With limits determined by the standard deviation of multiple pure samples, it would be easy to classify samples as “within acceptable limits” or “rejected” with a rapid measurement. The “suspected of adulteration” lavender A-TEEM contour is subtracted from the high-quality lavender sample A-TEEM, showing how different these contours are, (Figure 4).

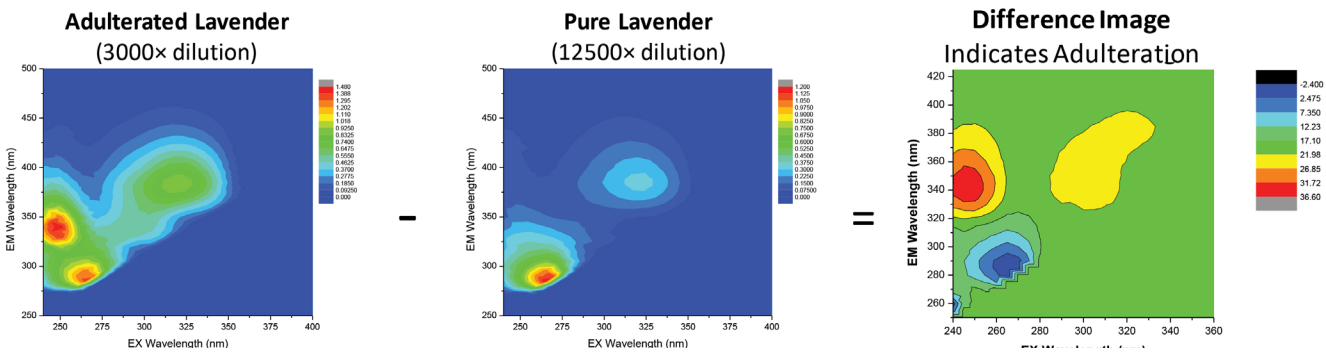

Figure 4: Subtraction of adulterated lavender essential oil A-TEEM from pure lavender essential oil A-TEEM to produce difference image describing areas of adulteration.

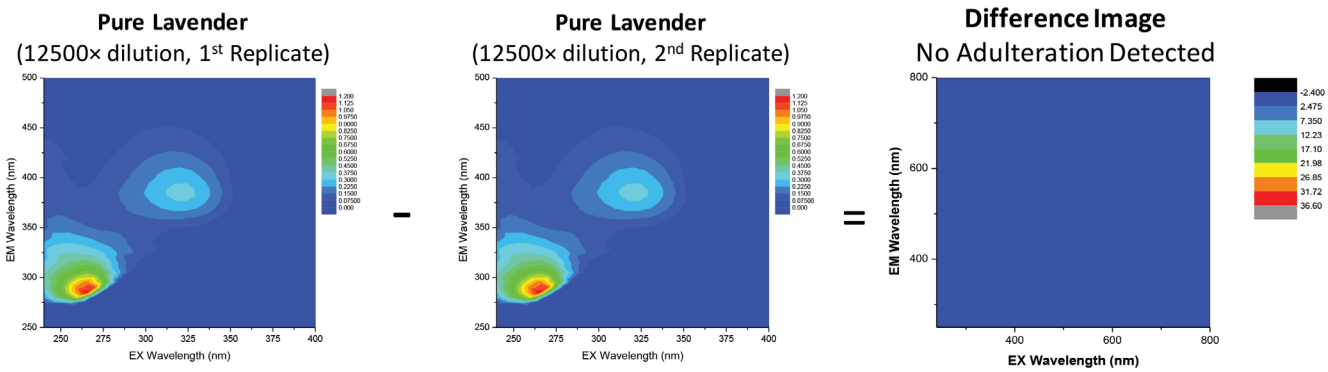

Figure 5: Subtraction of two replicate A-TEEMs of pure lavender essential oil to produce image denoting the lack of adulteration.

In contrast, when the A-TEEM contours of two replicates of the pure lavender essential oil are subtracted, the resulting difference EEM has a net signal of near zero (Figure 5). This demonstrates the capacity of A-TEEM to be used for rapid qualitative screening, to detect adulteration or contamination in essential oil samples quickly and efficiently.

In addition to the A-TEEM analysis, both lavender essential oil samples were also analyzed by GC-MS. The chromatogram of the high quality oil has strong peaks at retention times of 13.18 min, and 17.55 min (Figure 6a). The identity of the compounds associated with these peaks is linalool and linalyl acetate, respectively, and account for 63% of the total chromatogram peak area. Importantly, these species represent the two most predominant components found in pure lavender essential oil. For the oil sample suspected of adulteration, the two major peaks corresponding to linalool and linalyl acetate were also present but made up a much lower overall percent (31% versus 63%) of the sample components (Figure 6b).

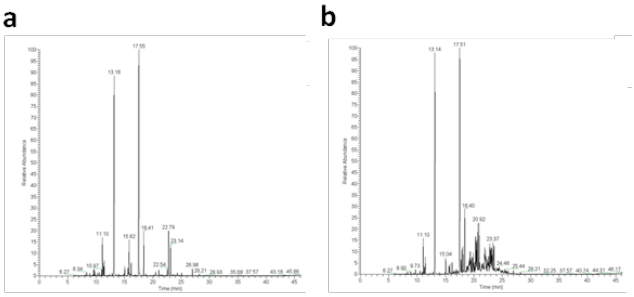

Figure 6: GC-MS chromatograms of pure lavender essential oil (a) and adulterated lavender essential oil (b).[1]

[1] GC-MS chromatograms and testing provided by: James Kababick, Chanze Jennings, Stacy Wise, Flora Research Laboratories, LLC, Grants Pass, OR

Additional smaller peaks are evident in the chromatogram. The addition of peaks not seen in the pure sample, along with the decreased contribution of linalool and linalyl acetate components indicate significant adulteration, showing nice agreement between A-TEEM spectroscopy and the GC-MS results.

Conclusions

Verifying the quality of lavender essential oil is crucial to ensure it is safe for its intended use, and to ensure consumers aren't being deceived into buying inferior products. Two samples of lavender essential oil with identical label claims of "Pure", one from a supplier that tests for quality and one that does not, were evaluated with A-TEEM spectroscopy and GC-MS. The GC-MS method was 35 minutes long, with an approximate per-measurement cost of $600. The A-TEEM method took 4 minutes, with an approximate per-measurement cost of $125. This quick yet sensitive method was able to detect adulteration, demonstrating its potential to screen samples and assess the purity of lavender essential oils in a timely and cost-effective manner.

It is anticipated that A-TEEM spectroscopy will be useful for a variety of natural products applications, not just essential oils. It provides a rapid approach for the detection of adulteration, overcoming limitations of existing approaches. In addition, A-TEEM has shown promise for quantification of compounds, with results comparable to chromatography.

References

Shulha, O. (2023). English Lavender Essential Oil Laboratory Guidance Document. Oxford, MS: ABC-AHP-NCNPR Botanical Adulterants Program.

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