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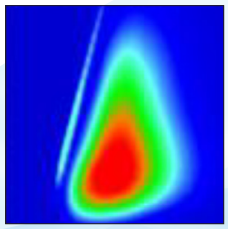
Fluorescence

Rapid Quality Assessment of Essential Oils using Fluorescence Excitation-Emission Matrix Spectroscopy



Application Note
Life Sciences
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Introduction

Essential Oils (EOs) are volatile essences extracted by physical means (pressing and distillation) from a whole aromatic plant or part of a plant. Due to their beneficial properties (antimicrobial, antiviral, anti-oxidative, antifungal…), EOs are formulated into many consumer products including perfumes, creams, bath products, and household cleaning substances, as well as being used for flavoring agents in food and beverages [1].

The International Organization for Standardization (ISO) sets standards for analysis methods and specifications for EOs through its technical committee - TC54. Chromatographic methods (GC, HPLC) dominate for the analysis of EOs. Although well established and accepted, chromatography methods are time consuming (10–30 minute methods are common) and can be expensive on a per measurement basis, requiring the use of mobile phase, standards, and columns. There is demand for rapid and inexpensive methods for quick survey scans, and optical spectroscopic techniques come into play, depending on the requirements of the measurement. Recent studies have reported the use of fluorimetry, less common than Raman or NIR, as a rapid and less expensive alternative [2] that is suitable for the characterization, discrimination, and QC of EOs. For applications where it is helpful to discriminate very similar compounds, a 3D implementation called fluorescence Excitation Emission Matrix (EEM), has been used. An EEM is created by scanning excitation and emission wavelengths, the results of which are displayed graphically as a contour against fluorescence intensity for each excitation emission pair. EEMs are used for a variety of applications where multi-component analysis is required and are often referred to as providing a molecular fingerprint for many different types of samples [3].

The origin and purity of EOs is an important quality attribute, as products sourced from a preferred region, or with a preferred composition, can be significantly more valuable. Because of the high value associated with these specific samples, counterfeiting can be a problem, with lower quality product substituted for, or mixed in with, the authentic product. Here we investigate an EEM-based approach for the rapid differentiation and classification of EOs sourced from different regions, and for samples spiked with an adulterant. We used Duetta™ (Figure 1), a two-in-one spectrometer, for these experiments. Duetta simultaneously measures UV-Vis-NIR absorbance and fluorescence simultaneously and can collect EEMs. These highly discriminating molecular fingerprints can be used to rapidly screen samples for authenticity. We propose that this technique could be used for rapid screening, identifying samples for further analysis if differences from one sample to another are detected.

Figure 1: Duetta Fluorescence and Absorbance spectrometer.

Methods and Results

Characterization and classification: Different providers

Three different EOs brands from three different countries of origin have been characterized in this study. All the samples were extracted by steam distillation and have been analyzed undiluted at room temperature in a 1-cm path quartz cell. Operational details of the Duetta spectrometer can be found elsewhere [4]. The excitation and emission wavelength ranges, integration time and slits width have been adapted depending on the samples' emission properties (i.e., intensity, spectral range) (Figure 2). Total acquisition time for every acquisition was < 15 sec.

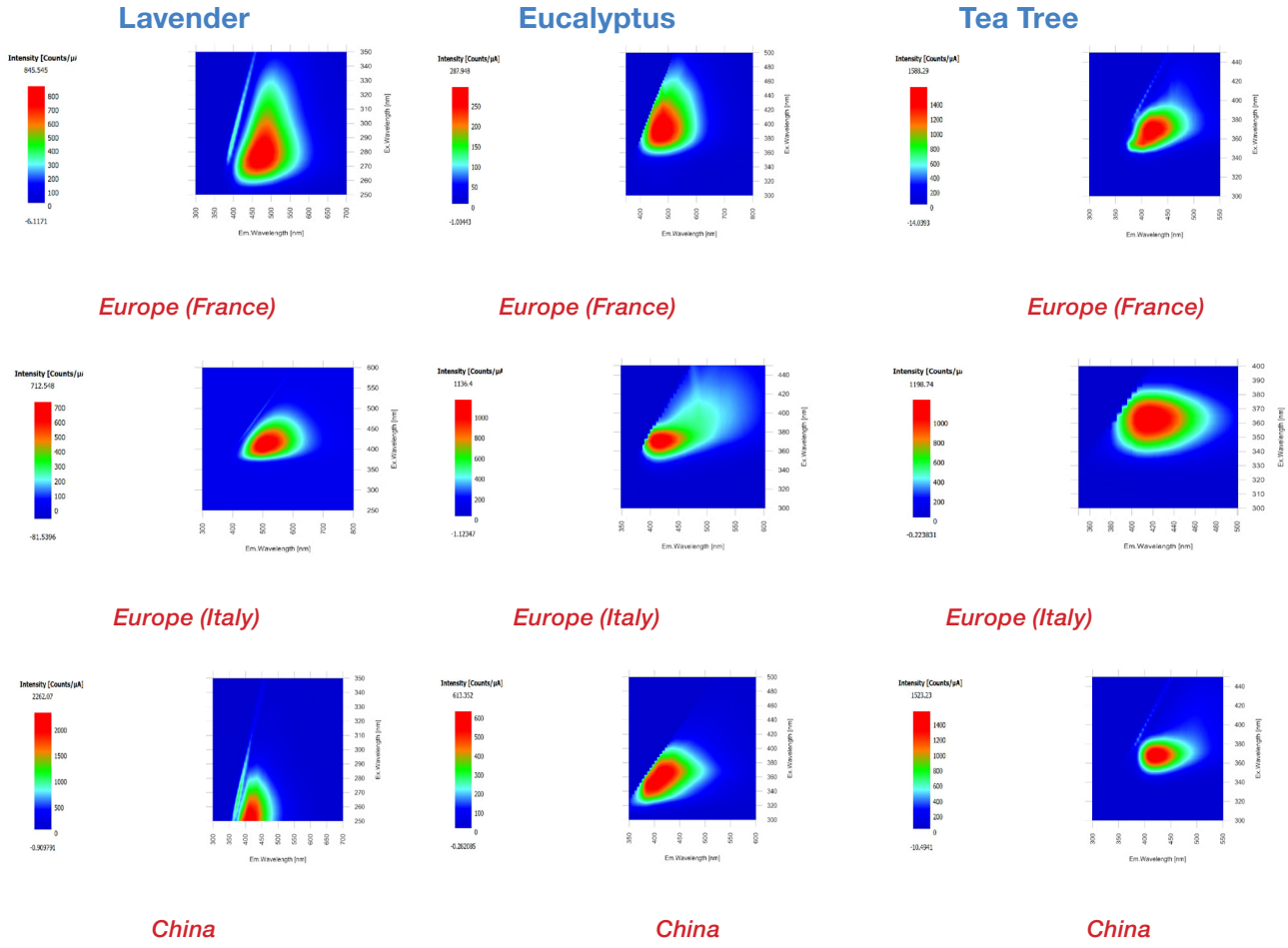

Figure 2: EEMs contour plots for pure component EOs. Note that the EEMS for the different EOs and different countries of origin are unique.

Most of the EOs present a fluorescence emission activity in the range 360-500 nm. This similarity is due to fluorophores that are common to the three EOs (Figure 3):

- Terpenoids - the largest and most diverse class of volatile organic compounds
- Phenols and flavonoids - aromatic compounds that account for most of the antioxidant activity in plants and plant products.

Despite the overall similarities between them, the EOs evaluated here were easily distinguished from each because of the sensitivity of fluorescence spectroscopy. The EEM profile for each is unique, a distinct molecular fingerprint resulting from that specific sample, and provides a method to quickly distinguish between species and origin.

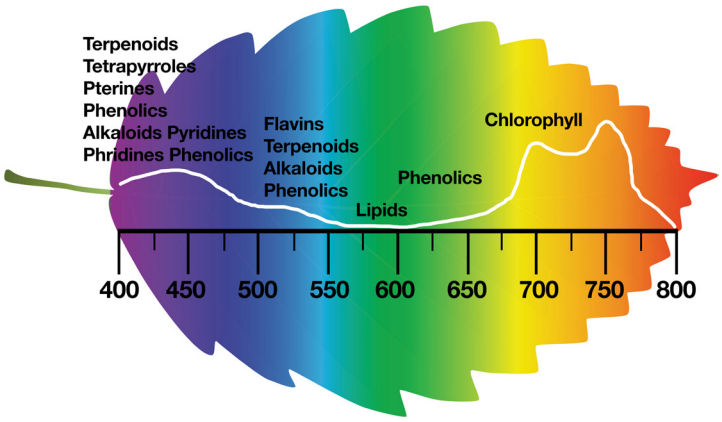

Figure 3: Fluorescence spectrum of a typical green leaf, with the main plant compounds [5].

Adulteration

There are several strategies for adulteration of EOs: The addition of volatile compounds, such as benzyl alcohol, and nonvolatile substances such as vegetable oils (i.e. castor oil, palm oils or another less expensive essential oils). The detection of volatile adulterants is easily detected by GC/MS/MS, whereas non-volatiles are less likely to be discovered. Producers of EO products, therefore, need to employ a multi-pronged approach to ensure purity. To test the capability of Duetta to distinguish between pure and adulterated lavender, we mixed the lavender and eucalyptus (both from brands sourced from France) in a 50-50 by volume mixture. The mixture EEM (Figure 4) results in a new fluorescence fingerprint that incorporates the eucalyptus character, with a broad major contour centered between 370-450 and 400-600 nm in the excitation-emission spectral range, instead of 260-340 and 400-570 ranges of the pure lavender.

Figure 4 shows that in a visual, qualitative assessment, the EEMs profile distinguishes pure lavender from the adulterated sample.

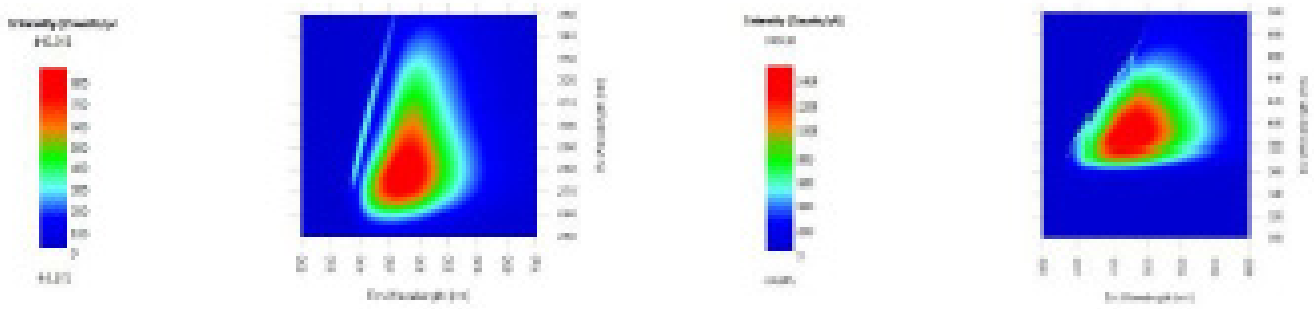

Figure 4: EEMs contour plots for pure lavender (left) and French lavender-eucalyptus mixture (right).

Conclusions

The Duetta was used to collect Excitation-Emission Matrices that distinguish different species of essential oils, as well as the same species by region of origin. A 50/50 mixture of lavender and eucalyptus demonstrated the ability of the approach to qualitatively identify an adulterant. Here are three reasons why the EEM method should be used:

Safety & Value:

- Adulteration detection: EOs are used in human and veterinary medicine. The most common way EOs are adulterated is by adding individual chemicals to the oil. The formulation of medicinal products with counterfeit and adulterated EOs can present a serious health risk for consumers [6].
- Characterization of pure EO samples by origin: EOs from specific origins can be very highly valued. Unethical suppliers may try to pass inferior product.

Efficacy:

- Characterization and classification depending on the botanical origin: Variety and season/conditions of cultivation may have a significant effect on the extraction yield, qualitative and quantitative profile of EOs.
- Quality control: The quality and the price of the EOs depend on their qualitative and quantitative composition. Various factors such as growing conditions, harvest time, method of extraction can affect the quality of the oils. This step has an essential role to ensure product quality.

The EEM method presented here can be used as a rapid screening technique to qualitatively assess sample quality. This provides an alternative to expensive and slow GC-MS methods. If differences are noted, more thorough analyses can then be performed.

For quantitative work, and for industrial QC/QA implementations of EEM (A-TEEM) spectroscopy, the HORIBA Aqualog A-TEEM spectrometer is highly recommended. Consult with your HORIBA representative for more details.

References

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