

Application Note

Keywords

- Exhaust emissions
- NO_x, SO₂ and NH₃
- Emission sensors

Techniques

- Absorbance
- UV spectroscopy

Applications

- Emissions monitoring
- Exhaust gases detection

Lavender Oil Adulteration

Analyzing Lavender Oil from Flower to Fluid



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Abstract

Essential oils have been used for thousands of years in many cultures for medicinal and health purposes, with uses ranging from aromatherapy to household cleaning to holistic medicine. Essential oils are produced by extracting the desired component from plant material, which is a costly and labor-intensive process. This makes these oils prime candidates for adulteration.

Some companies choose to add other chemicals to dilute the essential oil and extend how far the pure essential oil goes. Sometimes these companies will label the new solution as a “blend,” but many times manufacturers will not, leaving consumers with a product that is less pure than stated. Common additives used in dilution are fatty oils, alcohols, other similar oils, surfactants, and emulsifiers.

The authentication of essential oils is important, not only for quality control for producers but for consumer protection ^[1]. With essential oils, home remedies and holistic medicine becoming more popular, adulterated products are becoming increasingly prevalent. An adulterated essential oil can cause harm to the user whether it is for topical or internal use, especially if it causes a rash or allergic reaction.

The focus of this application note is the detection of adulteration in lavender essential oil using an attenuated total reflection infrared spectrometer, the **Ocean MZ5**. Lavender oil is one of the best-selling oils and most adulterated ^[2]. The Ocean MZ5 focuses

on a portion (1818-909 cm⁻¹) of the fingerprint region (~1500-500 cm⁻¹), which gives a unique spectrum for each compound [3]. Combining this region with the use of chemometrics, a powerful tool utilizing multivariate statistical analyses and data transformations, provides even greater analysis capabilities. It was determined that the limit of detection (LOD) of lavender in sweet almond oil is 6.74% and the LOD of sweet almond oil in lavender is 7.66%.

Background

Essential oils are said to have a variety of uses and health benefits, with lavender oil being a popular choice among essential oil users. Ancient Romans and Egyptians used lavender as perfume, for bathing, and cooking. Users and manufacturers of lavender oil claim it provides anxiety relief, restful sleep, reduction of skin blemishes and irritation, and scent and flavor benefits [4]. *Lavandula angustifolia* (formerly *L. officinalis*) is stated as “true lavender” and is the top choice for lavender essential oils. Many claim “true lavender” has the most medicinal properties and is sweeter scented compared to other varieties. It was originally called English lavender because it was used as the basis of England’s lavender oil industry in the 1700s.

In this study, the Ocean MZ5 was used along with chemometrics, a multivariate analysis method. Rather than using a single peak on the spectrum to produce a calibration curve, which is typical of absorbance measurement analysis, chemometrics uses the whole spectrum. Several reasons this type of analysis is used include the complexity of the sample, spectra that may have baseline shifts, and to account for more nuances in the whole spectrum.

Chemometrics allows small differences in the spectra to become more distinct through preprocessing transformations, along with multivariate statistical analyses. It allows the user to quantify, classify or discriminate samples. Quantification tells how much of a certain substance is in a sample, classification gives the identity of the sample, and discrimination says whether a sample is comparable to a standard. Two common methods of chemometric analysis, used in this experiment, are principal component analysis (PCA) and partial least square regression (PLS).

Chemometrics works by taking a training set to create a model and a test set to validate this model. When a test set is not available, cross-validation (testing the model with spectra taken from the original data set) will be used to validate the model. When it comes to complex samples, where traditional spectroscopic analysis may fail, chemometrics shines. In measurements where one peak may not define the sample, the whole spectrum can be used. The information from the spectra are extracted in a way that independent, orthogonal, factors (also referred to as principal components) are found to account for the most variation in the data. Each factor has a loading based on where the most important or influential data lies on the spectra. From this loading a score is determined, which is the contribution of each loading. This occurs for every spectrum, so each will have a score attributed for each loading.

Experimental

Nature’s Truth 100% Pure Lavender Essential Oil (*Lavandula angustifolia*, formerly *L. officinalis*) was diluted with Nature’s Truth 100% Pure Sweet Almond Oil – a fatty oil. Nature’s Truth verifies its oil purity using GC-MS techniques. Solutions were prepared in 5 mL vials using a micropipette to produce a range of 0%-100% lavender oil in sweet almond oil, in increments of 20%. Two sets of 6 samples were made for the calibration set and a test set of 5 samples was made to project onto the model.

Set Concentrations	
Calibration Set	Test Set
% Lavender	% Lavender
0	10
20	25
40	50
60	75
80	90
100	

Table 1. Calibration set and test set concentrations of lavender in sweet almond oil (v/v).

Each of the 12 calibration samples and 5 test samples was analyzed using the Ocean MZ5. Standard device settings were used: 8 Hz pulse rate, 100% light intensity, 40 warm-up scans and 400 scans per measurement. One hundred measurements were taken per calibration sample and 30 measurements were taken per test sample.

Each sample was pipetted onto the surface to cover the MZ5 crystal. Initially, 30 air background measurements were taken, followed by 100 sample measurements. The crystal was then wiped of the sample and cleaned using isopropyl alcohol (IPA); the process was repeated for all samples. The air background measurements were used with the sample measurements to calculate the absorbance of each sample.

Results and Discussion

Initially, the absorbance spectra of the lavender oil/sweet almond oil solutions were plotted to see if there were distinct differences in the spectra or if it was immediately obvious that some preprocessing needed to be performed.

Upon visual inspection, the spectra appear to have differences that depend on concentration, seen in **Figure 1**. To determine if separation and clustering of the data is possible, principal component analysis (PCA) was performed, shown in the **Figure 1** inset. PCA is a common method used to determine grouping of data. While each of the concentrations is separated from one another, individually they are not tightly clustered. The groups of the same concentration should be clustered together, rather than in two separate clusters like they are. There was no change in experimental parameters, concentrations, or any other variable so this is likely due to baseline shifting while taking the measurements over a few days.

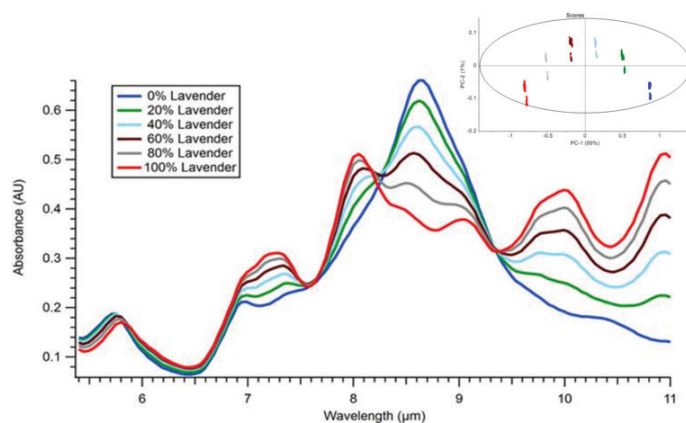


Figure 1. Sweet almond oil in lavender oil absorbance, inset. Scores plot of PCA analysis shows poor clustering of data.

To account for baseline shifting a standard normal variate (SNV) transformation was performed on the spectra, to investigate if further clustering of the already separated data could be achieved. SNV is a pre-treatment that is used often with many infrared spectra to adjust for these effects. SNV is applied to every spectrum individually, where the average and standard deviation of all the data points are calculated, then each data point of the spectrum is subtracted from the mean and divided by the standard deviation. After this, the spectra are centered and scaled, shown in **Figure 2**. The data is clustered tighter together after the SNV transformation, shown in **Figure 2** inset.

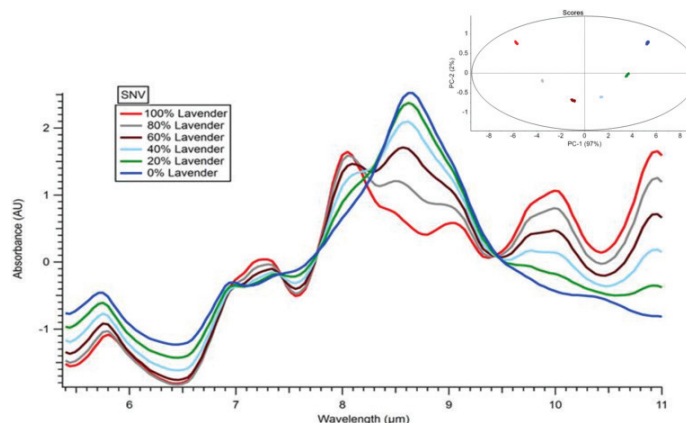


Figure 2. Absorbance spectra after SNV transformation, inset. PCA scores plot after SNV transformation.

After doing a PCA analysis, a partial least square regression (PLS) model was created to quantify the data. The model was trained with the SNV transformed data, which gave good clustering of the data as it did with the PCA analysis, shown in **Figure 3** below.

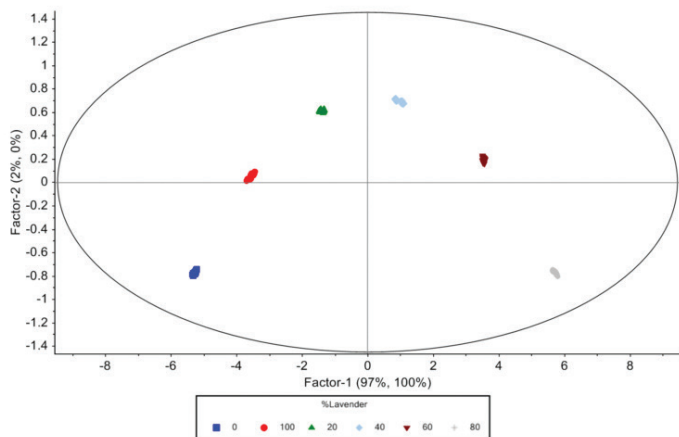


Figure 3. Calibration scores plot of PLS model with concentrations ranging from 0%-100% lavender in sweet almond oil.

The PLS model was initially estimated at 7 factors and found to be optimal at 1 factor, accounting for most of the explained variance. By expanding to the second factor there was only an explained variance gain of 0.1905%. Including additional factors does not improve the model.

Explained Y Total Variance	Factor 1	Factor 2	Factor 3	Factor 4
Calibration	99.6447	99.8352	99.8775	99.9093
Validation	99.6423	99.8339	99.8722	99.8992
	Factor 5	Factor 6	Factor 7	
Calibration	99.9176	99.9229	99.9251	
Validation	99.9008	99.8982	99.8964	

Table 2. Explained variance of PLS model factors

Cross validation of the data yielded a high R^2 value of 0.9964 and root mean square error of the cross validation (RMSECV) of 2.0359% (v/v), shown in **Figure 4**. Then this model was used for prediction of the lavender content of the test sample set.

The LOD was determined by using the formula: $LOD = 3.3 a / m$. Where a is the standard deviation and m is the slope of the calibration curve. The LOD of this model is presented in **Table 3** as lavender in sweet almond oil and sweet almond oil in lavender, which is done by changing whether the model is trained with lavender or sweet almond oil as the represented component.

Sample	LOD
Lavender in Sweet Almond Oil	6.74% Lavender
Sweet Almond Oil in Lavender	7.66% Sweet Almond Oil

Table 3. LOD of PLS models

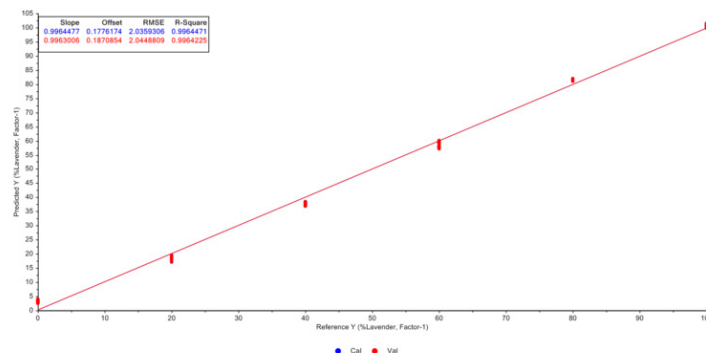


Figure 4. Predicted vs Reference calibration curves of calibration model and internal validation model

The test sample set projection on the model produced a line with a high R^2 value of 0.9893 with a RMSEP (root mean square error of prediction) of 3.0874%, shown in **Figure 5**. While the RMSEP was around 3%, deviation from the actual and predicted values ranged from 1%-2.5%.

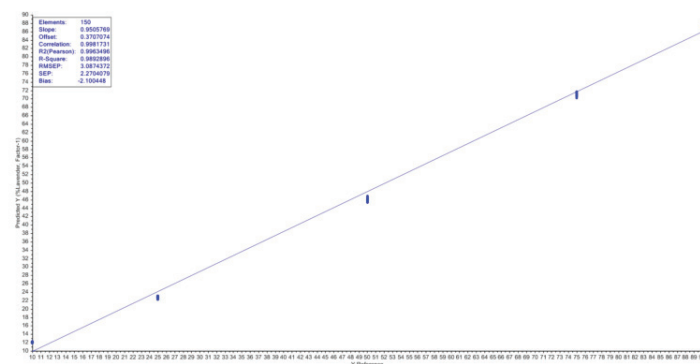


Figure 5. Predicted vs Reference of PLS calibration model with test sample set used for prediction

Conclusion

The Ocean MZ5 ATR spectrometer uses the IR fingerprint region to discriminate among different compounds due to the unique bands for each compound. Coupled with chemometric analyses and spectral transformations, the differences between different compounds and similarities between the same compounds become even more distinct, as seen in this study. It was determined that the limit of detection (LOD) of lavender in sweet almond oil is 6.74% and the LOD of sweet almond oil in lavender is 7.66%. 🌸

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