Application Note AN-RS-009

Identification and checking of fatty acids in functional foods and cosmetics

This Application Note describes the utilization of the Metrohm Instant Raman Analyzer Mira M-3 for identification and verification of fatty acids, similar to those found in cosmetics or nutraceuticals. Nutraceuticals are food-derived products that claim to provide extra health benefits in addition to basic nutritional value and can be found in gel caps. As the personal health industry moves toward natural homeopathic treatments, many new products are emerging on the market that report benefits to supplementation of diet with vitamins and fatty acids, such as oils that are sources of vitamin E but do not raise LDL ("bad") cholesterol. Some nutraceuticals are regulated by the FDA, while others are not. Regardless, it is important to manufacturers that their products meet internal and external regulations. Determination of ingredient identity and purity are essential to product quality, and inspection of ingredients prior to the start of the manufacturing process will prevent costly time delays and substandard product quality.

INTRODUCTION

Fatty acids used in production need to be verified during production processes. Similarities in fatty acids can make identification of the exact fatty acid through Pearson's correlation algorithm used for identification difficult; however, verification with a p-value algorithm produces a more confident method to ensure the correct material is used in manufacturing. The Metrohm Instant Raman Analyzer 3 (Mira M-3) is a handheld Raman spectrometer designed for rapid, nondestructive identification and verification of samples. The process of identification of samples involves measuring a spectrum of the sample and comparing it with existing spectra in a library. The result is then displayed with a Pearson's correlation. The verification of samples is performed with a training set of the spectra that contains the accepted variability between different samples of the same material. The training set is analyzed with Principal Components Analysis (PCA) and reported as a percent likelihood that the sample measured is within a confidence level set by the operator. Typically, a 95% confidence level is used for material verification. While both identification from a library and verification with a training set are useful, verification is able to detect very small differences in samples. The fatty acids and fatty alcohol discussed in this application note will be lauric acid ($C_{11}H_{23}CO_2H$), myristic acid ($C_{13}H_{27}CO_2H$), palmitic acid ($C_{15}H_{31}CO_2H$), stearic acid ($C_{17}H_{35}CO_2H$), and stearyl alcohol ($C_{17}H_{37}OH$). Figure 1 shows the spectra of these materials and the spectral similarities, illustrating the difficulty of differentiation on correlation alone.

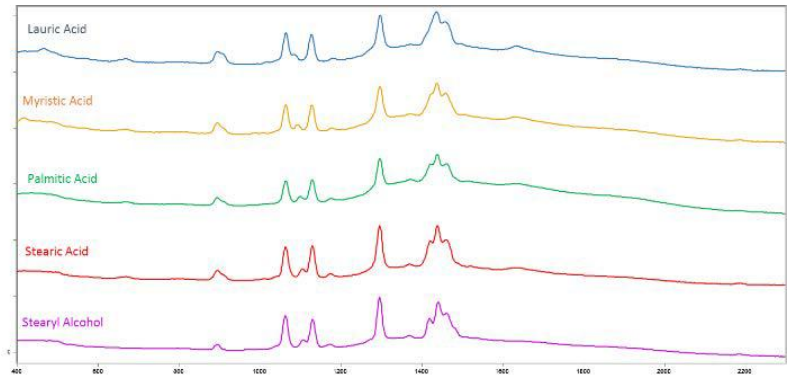

Figure 1. Raman spectra of the fatty acids and fatty alcohol discussed in this Application note

EXPERIMENTAL

Creating an operating procedure (OP)

In MiraCal software, create an OP to build the library, select the Operating Procedures tab and create a new OP "Fatty Acids". The parameters are set to laser power 5, average of 1, and auto integration time. Acquire a spectrum of each sample with the OP, carefully name each sample, and synchronize the data with the MiraCal software database.

Creating and testing the fatty acid library

From the samples that were saved using the "Fatty Acid" OP, the fatty acid library can be created. Select the Libraries tab and name the new library "Fatty Acid Library". Add the previously collected samples to the "Fatty Acid Library" and save it. Next, create a new OP with the name "Library Testing" and set the parameters to laser power 5, average of 1, and auto integration time. With the Evaluations tab selected, check the identification box and select the "Fatty Acid Library". Save the "Library Testing" OP and synchronize it with your system. The system can now be used to match samples against the "Fatty Acid Library". An example of match scores for each fatty acid sample is illustrated in Table 1.

Creating and testing the fatty acid training set with p-value

Select the "Fatty Acids" OP that was created in the previous experiment, and proceed to collect ~20 spectra of each fatty acid sample. Once finished, connect the instrument to the MiraCal software and synchronize the data to the database. The next step is to create a training set for each sample. Select the Training Sets tab in the software, and proceed to create new training sets for each material by entering the sample name as the training set name and adding the ~20 spectra that were collected in the previous step. Once all 5 training sets have been created and saved, the next step is to create new OPs that correspond to each training set. All five OPs will have the same acquisition parameters: auto integration time selected, laser power 5, and average set to 1. In the Evaluation tab of the OP, check the Verification box for each OP, and add the corresponding training set by pressing the "Training Set" button.

Once this is finished, save each OP and synchronize the software database to add the OPs to the system.

Now measure a spectrum of each sample against each OP. The Pass/Fail results are recorded in Table 2.

RESULTS AND DISCUSSION

As we saw previously, simple library matching (Pearson's correlation) does not always accurately identify the correct material when other similar materials are present in the library. The match scores of similar materials may only differ by 0.01–0.03 Hit Quality Index (HQI), which is difficult to interpret and lowers the confidence of the analysis (Table 1).

Table 1. Pearson correlation values between different fatty acids and fatty alcohol tested in the Application Note.

Sample	Pearson correlation value	Match sample
Palmitic acid	1.00	Palmitic acid
	0.98	Myristic acid
	0.98	Stearic acid
Stearyl alcohol	1.00	Stearly alcohol
	0.97	Stearic acid
	0.93	Palmitic acid
Lauric acid	1.00	Lauric acid
	0.98	Myristic acid
	0.95	Palmitic acid
Myristic acid	1.00	Myristic acid
	0.98	Palmitic acid
	0.98	Lauric acid
Stearic acid	1.00	Stearic acid
	0.97	Palmitic acid
	0.95	Stearyl alcohol

Verification measures the sample against the selected training set, and if the sample falls within that training set, there is a positive result ("Pass"). If the sample falls outside of the training set, there is a negative result ("Fail"). By creating verification models for each of the fatty acid samples and testing each model against each sample, we can see that the instrument is always able to accept, or "Pass", the correct sample, and reject, or "Fail", samples that are similar yet different. Additionally, the verification result is easy to interpret (Table 2). For example, palmitic acid passes with a 33.1% confidence that is within the 95% confidence interval set.

SAMPLES	TRAINING SETS				
	Palmitic Acid	*Stearyl Alcohol*	*Lauric Acid*	*Myristic Acid*	*Stearic Acid*
Palmitic Acid	PASS 0.331	FAIL 0.000	FAIL 0.000	FAIL 0.000	FAIL 0.000
Stearyl Alcohol	FAIL 0.000	PASS 0.628	FAIL 0.000	FAIL 0.000	FAIL 0.000
Lauric Acid	FAIL 0.000	FAIL 0.000	PASS 0.127	FAIL 0.000	FAIL 0.000
Myristic Acid	FAIL 0.000	FAIL 0.000	FAIL 0.000	PASS 0.494	FAIL 0.000
Stearic Acid	FAIL 0.000	FAIL 0.000	FAIL 0.000	FAIL 0.000	PASS 0.365

Table 2. Pass and fail results of different samples versus the training set

CONCLUSIONS

Identification is useful when identifying samples that exhibit large differences in spectra, and verification is useful when examining samples with similar material spectral features. When there is no prior knowledge of what the sample is, correlation is used to search a library of known materials to try to identify the unknown. When there is prior knowledge of what the sample is but the sample needs to be confirmed as authentic, verification of the sample with the p-value is best. The "Pass" and "Fail" results of verification give a more confident confirmation of what the sample is, whereas with identification, there is a potential for high match scores with samples that are very similar to each other.

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