Application Note AN-RS-009

功能性食品和化品中的脂肪酸

This Application Note describes the utilization of the Metrohm Instant Raman Analyzer Mira P 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. As the personal health industry moves toward natural homeopathic treatments, many new products report benefits to supplementing the 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 must be verified during production processes. Similarities in fatty acids can make identification of the exact fatty acid through Pearson's correlation algorithms difficult; however, p-value verification ensures that the correct material is used in manufacturing. MIRA P is a handheld Raman spectrometer designed for rapid, nondestructive identification and verification of samples. Identification of samples involves measuring a spectrum of the sample and correlating it with existing spectra in a library. The result is then displayed with a Pearson's correlation. 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.

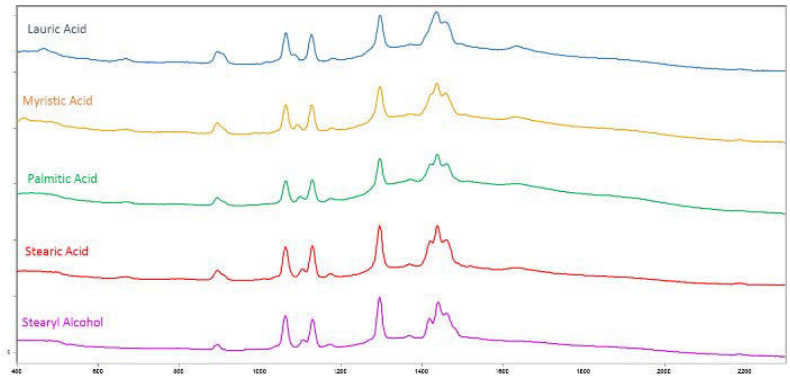

Figure 1. Raman spectra of the fatty acids and fatty alcohol discussed in this Application note

EXPERIMENTAL

Creating an operating procedure (OP)

In MiraCal software, 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, 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 PASS the correct sample and 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 spectral features. For unknown samples, correlation is used to search a library of known materials to try to identify the unknown. When a 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.

CONTACT

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CONFIGURATION

MIRA P Advanced

瑞士万通快速拉曼分析 (MIRA) P 是一款性能大的手持式拉曼光,可用于各材料的快速无定和,例如物有效成分和形。MIRA P 小而固,配了瑞士万通的ORS技。MIRA P 符合 FDA 邦法 21 章第 11 款的定

。Advanced Package 包含一个附加透,可用它直接分析材料或者在材料容器中分析(3b 激光器),有一个小管支架套筒用于分析玻璃小管中的本(1 激光器)。

MIRA P Basic

瑞士万通快速拉曼分析 (MIRA) P 是一款性能大的便携式拉曼光,可用于各材料型的快速无定和,例如物有效成分和助材料。尽管 MIRA P 体很小,但是其采用了固的,并且具ORS特色技。MIRA P 符合 FDA 21 CFR 第 11 部分的准要求。

使用 MIRA P Basic-Paket,用可以根据其需求 MIRA P 行整。MIRA Basic-Paket 是一款入套装,其包含了行 MIRA DS 所需的基本件。

基本套餐包含了 MIRA 校正/配件、USP 功能和用来在瓶子或袋子里行分析的 LWD 附件。激光防等 3B 的使用。

MIRA P Flex

使用 MIRA P Flex Package,用可以根据其需求 MIRA P 行整。Flex Package 包括了用来行 MIRA P 的所有基本件,无需采用附件。行需要一个采用附件。MIRA P Flex Package 包括了 USP 功能,用于校正/的附件和一根 USB 。以 3B 投入使用。