

P-test verification of Equate cold tablets and comparison with Alka-Seltzer

Producers of generic brands offer cosmetics, medicines and other goods in competition with name brands, often at a lower price point. This lower cost may reflect a lack of research, development, and advertising costs, but should never imply lower quality, especially in the case of over-the-counter drugs. As an example, Equate (a Walmart brand) effervescent cold tablets promise customers the same active ingredients in the same proportions and with

identical effectiveness as Alka-Seltzer, at a much lower price. This Application Note demonstrates that Raman spectroscopy can successfully verify that these competing cold tablets are not identical. The process of ingredient verification involves a p-test, which measures the acceptable variability of a sample spectrum, as compared to a representative training set.

INTRODUCTION

This application note demonstrates that Raman spectroscopy can distinguish between competing over-the-counter cold medicines. It also describes best practices for establishing a training set that will permit detection of very small differences between samples.

The Metrohm Instant Raman Analyzer (Mira P) is designed for the rapid, nondestructive identification and verification of samples.

Identification of an unknown sample involves comparison of its spectrum within a library of known compounds. This results in spectral matches based on similarity. Verification is sensitive to very small spectral differences and is used when the authenticity of a known sample must be confirmed. A p-test is a type of multivariate analysis used for verification, rather than identification. A p-test measures the acceptable variability of a sample spectrum, as compared to a representative training set. The reported value indicates the statistical confidence that the sample “belongs” to the training set. In this way, spectral data can be used to assess and compare the chemical similarity of generic and name-brand drugs. The Mira P is equipped with Orbital Raster Scan (ORS™) technology, which increases the interrogation area on the sample while maintaining high spectral resolution. This ensures that even inhomogeneous samples, such as effervescent cold tablets, can be verified with confidence.



EXPERIMENTAL

Training Set

The effectiveness of a p-value depends on the quality (or robustness) of the training set. A robust training set accounts for normal variations, unrelated to the chemical composition of the sample, which may be encountered in the course of verification. For example, multiple batches from the same manufacturer or batches from different producers should be sampled for the training set. Additional

variances such as ambient light and temperatures, differences in sample containers, and day-to-day instrument variability should also be included.

For this note, spectra were collected from different locations on each tablet in order to create a training set that represents the full inhomogeneity of the tablets. A minimum of twenty samples is required for statistical purposes.

The Mira P with an excitation wavelength of 785 nm and equipped with a short working distance (SWD) attachment was used to collect 60 different spectra: 20 each with high, low, and auto integration settings.

These spectra were processed using MiraCal software. The same operating procedure (instrument acquisition parameters) was used to establish the training set and for sampling of the tablets:

| | |
|---------------------|-----------|
| Laser Power | 5 |
| Auto integration | ON |
| Average | 1 |
| Smart tip | Allow All |
| Confidence interval | 0.95 |
| Match score | 0.85 |
| Library | USP |

METHOD

Equate effervescent cold relief tablets (EQ) were used to create training sets and operating procedures. These were used to determine the similarity of EQ and Alka-Seltzer Plus cold formula (AS). The hygroscopic

tablets were sampled out of the wrapper, which required fresh samples every half hour. Care was taken to acquire spectra from various areas of each tablet.

RESULT AND DISCUSSION

The measured p-values, which are well above the assigned confidence level of 0.05, validate the

robustness of the EQ training set. See **Table 1**:

Table 1. Verification of the EQ training set

| p-value | Result |
|---------|--------|
| 0.146 | PASS |
| 0.204 | PASS |
| 0.712 | PASS |
| 0.648 | PASS |

This indicates that the sample spectra fall within an acceptable level of variance, compared within the representative samples included in the training set.

Sampling Alka-Seltzer tablets and comparing them within the EQ training set provides interesting results:

Table 2. Comparison of Alka-Seltzer tablets with EQ training set

| p-value ORS ON | Result | p-value ORS OFF | Result |
|----------------|--------|-----------------|--------|
| 0.013 | FAIL | 0.533 | PASS |
| 0.008 | FAIL | 0.573 | PASS |
| 0.008 | FAIL | 0.197 | PASS |
| 0.180 | PASS | 0.010 | FAIL |
| 0.020 | FAIL | 0.010 | FAIL |
| 0.000 | FAIL | 0.056 | PASS |
| 0.000 | FAIL | 0.131 | PASS |
| 0.000 | FAIL | 0.082 | PASS |
| 0.000 | FAIL | 0.007 | FAIL |

The Mira P successfully verifies that EQ and AS tablets are not chemically equivalent, as the p-values in the first two columns of **Table 2** demonstrate. An additional experiment, where AS tablets are sampled with the ORS ON and OFF, demonstrates the effect of inhomogeneity on verification. When an inhomogeneous sample is tested with the raster off, each scan of the surface will verify only the

components present in a very discreet area. As the composition varies, so will the spectra. Sampling with the raster ON resulted largely in failed verification and very low p-values, but it often passed with the raster OFF. If the ingredients were different in identity, amount and relative distribution, all scans would be expected to FAIL.

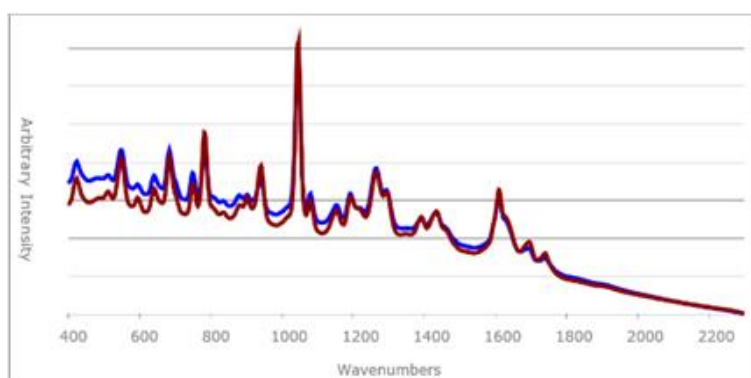


Figure 1. Raman spectra of EQ and AS samples

The results in the third and fourth columns of **Table 2**, combined with close comparison of the spectral peaks seen in **Figure 1**, suggest that the ingredients in each brand are qualitatively very similar. However, the

actual distribution of the active ingredients is different between the brands, resulting in failed verification. This demonstrates the contribution that the Mira P ORS technique makes to sample verification.

CONCLUSION

Verification with p-values using a Mira P handheld Raman spectrometer quickly, conveniently, and successfully determines that the identity and proportion of active ingredients in two competing

over-the-counter medications are similar, but not identical. In addition, the Mira P is sensitive enough to detect differences in homogeneity between the brands.

CONTACT

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CONFIGURATION



MIRA P Advanced

Der Metrohm Instant Raman Analyzer (MIRA) P ist ein leistungsstarkes Raman-Handspektrometer, das für eine schnelle zerstörungsfreie Bestimmung und Verifizierung verschiedenster Materialien, z. B. pharmazeutische Wirkstoffe und Arzneimittelträger, eingesetzt werden kann. Trotz seiner geringen Grösse ist der MIRA P sehr robust und verfügt über eine hocheffiziente Spektrographkonstruktion, die mit unserer einzigartigen Orbital-Raster-Scan-Technologie (ORS) ausgestattet ist. MIRA P erfüllt die FDA-Vorschriften 21 CFR Part 11.

Das Advanced Package umfasst eine Aufsatzlinse, mit der Materialien direkt oder in ihren Gebinden analysiert werden können (Laserklasse 3b), sowie einen Vial-Halter-Aufsatz zur Analyse von Proben, die sich in Glasvials befinden (Laserklasse 1).