Application Note AN-RS-010

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.

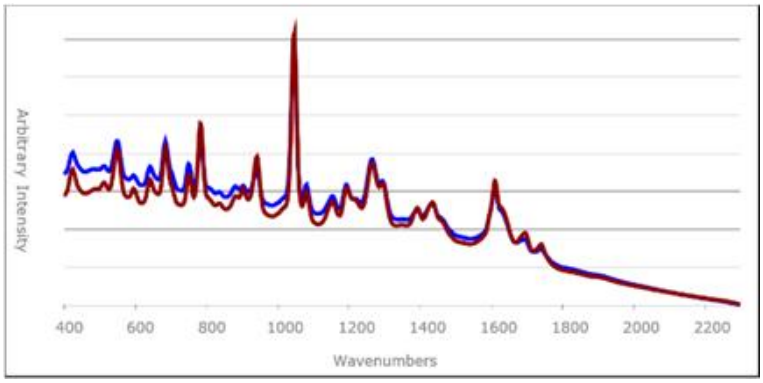

Figure 1. Raman spectra of EQ and AS samples

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.

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CONFIGURATION

MIRA P Advanced

Le Metrohm Instant Raman Analyzer (MIRA) P est un spectromètre Raman portable performant qui s'utilise pour les déterminations rapides et non destructives et le contrôle des matériaux les plus divers, comme les principes actifs pharmaceutiques et les excipients. De très petite taille, le MIRA P est pourtant très robuste et dispose d'une structure de spectrographe haute efficacité, équipée de notre technologie « Orbital Raster Scan » (ORS) inédite. MIRA P satisfait aux prescriptions FDA 21 CFR partie 11.

Le Advanced Package comprend une lentille avec laquelle les matériaux peuvent être analysés directement ou dans leur conditionnement (classe de laser 3b), ainsi qu'un support de flacon pour analyser les échantillons dans des flacons en verre (classe de laser 1).