

# Biologic Drug Identification at Fill and Finish

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## ABSTRACT

Pharmaceutical and biotechnology manufacturers must ensure the quality of materials - from incoming raw material through finished product. The Thermo Scientific™ TruScan™ RM Handheld Raman Analyzer delivers reliable material identity verification through sealed packaging in seconds, right at the point-of-need. With the optional Thermo Scientific™ TruTools™ embedded chemometrics package, users can build advanced, customized qualitative and quantitative methods for complex material analysis problems.

## INTRODUCTION

The rapid increase in demand for biopharmaceuticals presents manufacturers with unique challenges to ensure quality and regulatory compliance while maintaining supply and production capacity. Under GMP guidelines the identity of every container of concentrated commercial drug product and excipient raw material for use in a parenteral drug must be verified, therefore, each incoming bottle of commercial product must be tested in a QC lab before use in fill-finish production. For one pharmaceutical manufacturer and its contract manufacturing organizations (CMOs), this means several thousand bottles per year must be tested for just a single biologic product.

Fill-finish sites may not have the analytical labs with necessary testing equipment and technical resources to perform GMP-required verification testing of protein drug products. Consequently, these sites often ship protein product samples to QC labs for testing causing delays of several days in production and incurring additional costs.



Excipients such as buffers, salts, and sugars are rapidly tested and verified upon receipt in the warehouse using handheld Raman or NIR spectrometers. Traditionally, the verification test for biologic proteins is peptide mapping – a long-established workflow for protein identification using LC/MS.

This complex separations technique requires protein extraction and clean-up, enzyme digestion, one or more stages of liquid chromatography, and two phases of mass spectrometry before the final spectrum is matched against protein databases. Although a standard methodology, peptide mapping necessitates an analytical lab with qualified technical resources, entails extensive time for preparation, and introduces significant costs in solvents, columns, and analytical equipment.



With strong chemical specificity and highly reproducible results, portable Raman analyzers are an important technology in the pharmaceutical environment. Also beneficial to the industry, these instruments can measure aqueous samples and authenticate materials with little or no sample preparation anywhere in a pharmaceutical manufacturing plant. All these characteristics make handheld Raman analyzers such as Thermo Scientific™ TruScan™ RM well suited for biopharmaceutical manufacturing.

## DESIGN

A multinational biopharmaceutical manufacturer ran a feasibility study using TruScan RM with TruTools™ to ascertain if the handheld Raman spectrometer could identify different bulk biologic drug products. The biologics company wished to determine if the TruScan RM with TruTools add-on chemometrics package could:

- Discriminate between 6 different protein products (here designated Commercial Products A to F)
- Confirm the presence of a protein product, that is, distinguish between a therapeutic protein product and its placebo
- Differentiate between the placebos of various drugs
- Verify the identity of a protein product given the product's concentration

In this study, 6 different commercial protein products and 3 different placebos were analyzed using TruScan RM with TruTools. Protein products ranged in concentration from 20 mg/mL to 100 mg/mL.

As background to this feasibility study, the standard TruScan RM embedded multivariate residual analysis decision engine can effectively identify the majority of pharmaceutical materials directly. This standard verification approach is adaptive; it achieves a target signal-to-noise ratio for the sample while monitoring measurement uncertainty due to variables such as sample characteristics, instrument telemetry, and the sampling environment. The final probabilistic evaluation of the freshly acquired spectrum against the known reference spectrum is then presented as a straightforward pass or fail result. In this study, clear differences for one protein product (Commercial Product A) in structure, concentration, and placebo from the other protein products (Commercial Products B to F) were sufficient to differentiate it from the others in standard operation mode. To discriminate the other proteins, spectra acquired with the TruScan RM benefitted from additional spectroscopic modeling.

With the addition of TruTools chemometrics, TruScan RM can successfully characterize more highly similar or diluted formulations found in biologics manufacturing. Derivatized TruScan RM spectra display the presence of a protein at a concentration of 20 mg/mL versus its placebo (figure 1).

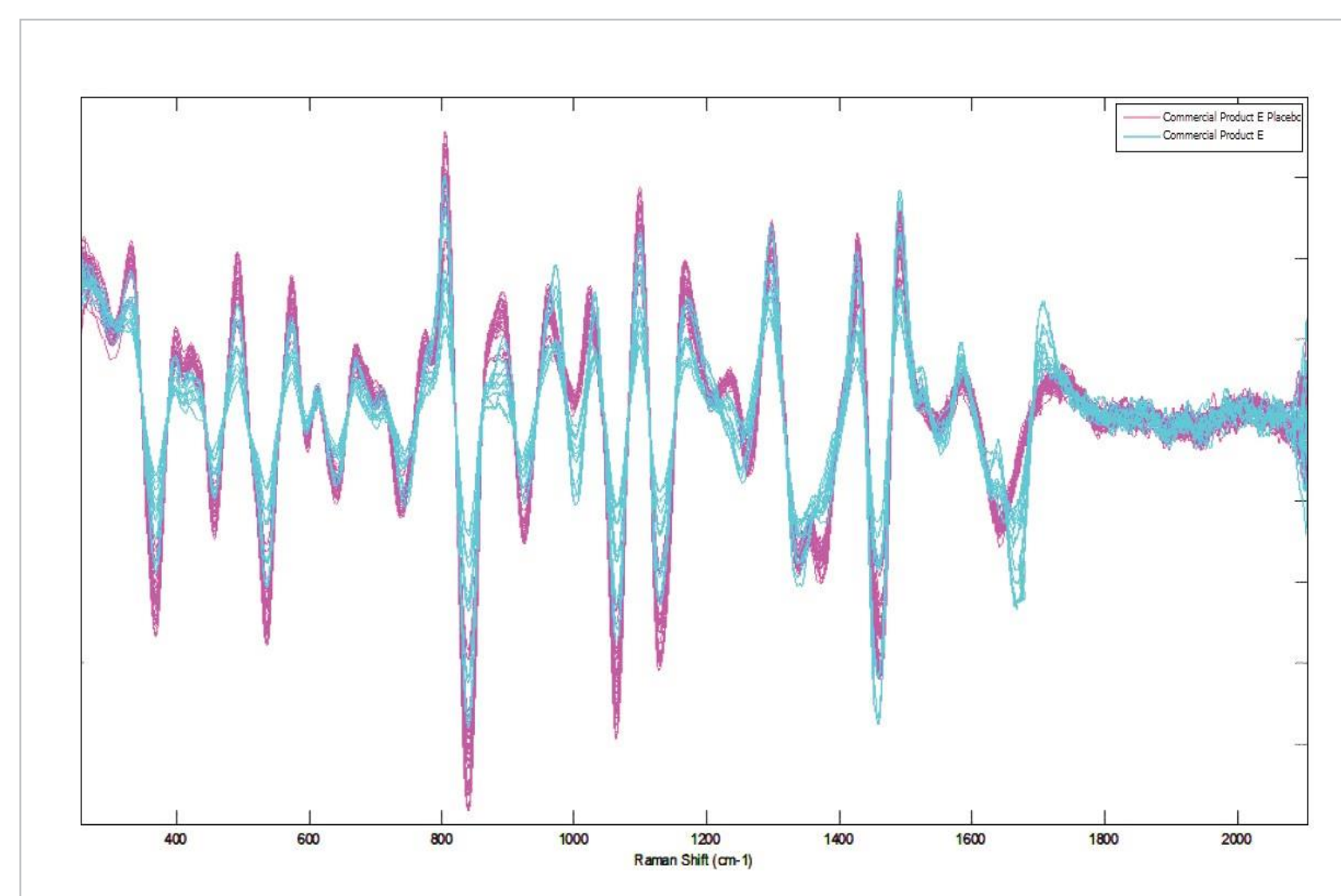


Figure 1. Preprocessed raw spectra show differences from the placebo related to the presence of an amide band from Commercial Product E

Using a PCA model built in TruTools, additional investigation of the data was performed and verification of the protein product and differentiation between the protein product and its placebo was achieved (figure 2).

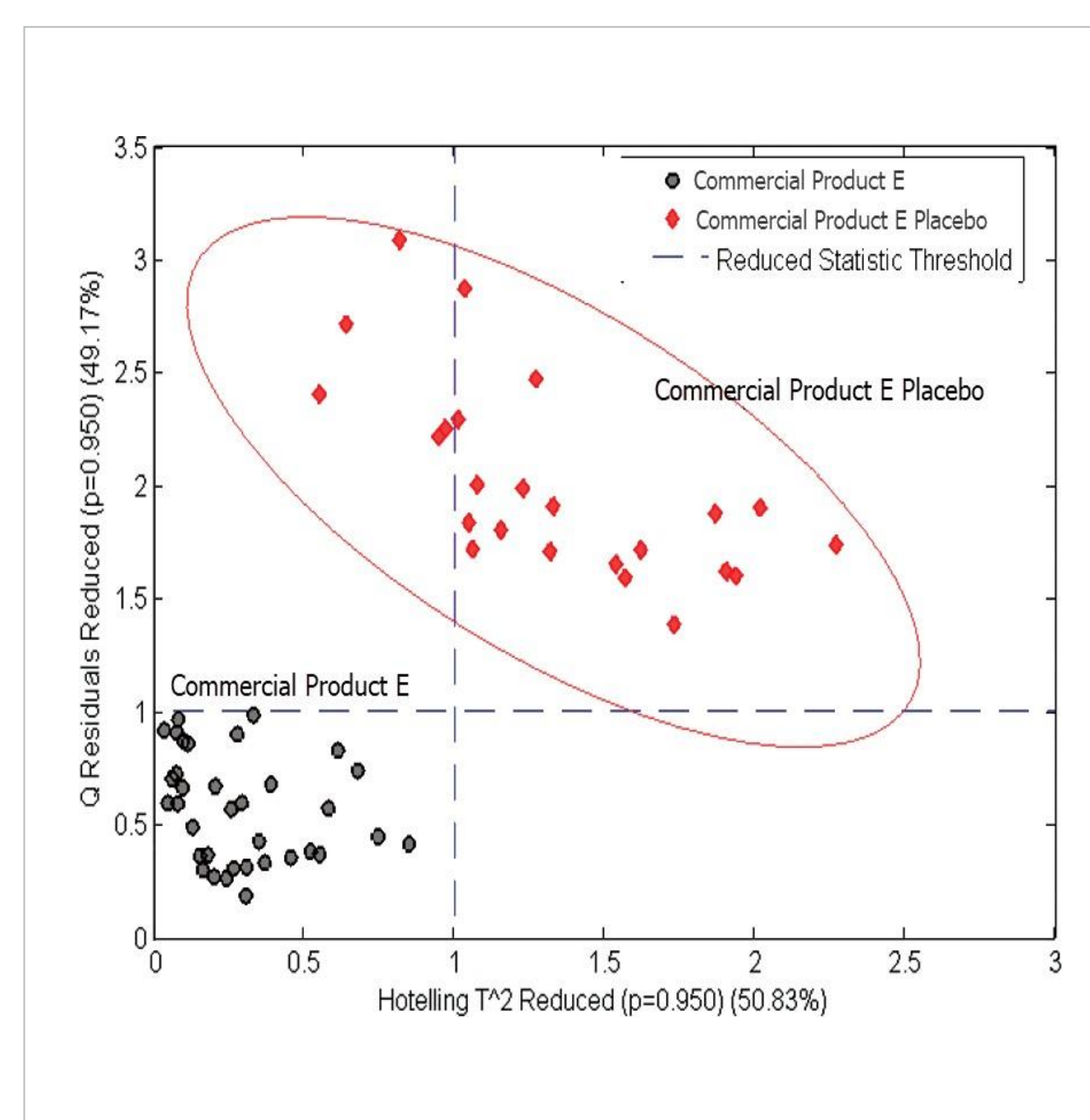


Figure 2. Q residual-T2 plot of PCA model built in TruTools showing good separation between Commercial Product E and its placebo

Thermo Scientific's TruTools is an add-on chemometrics package which runs natively on TruScan RM extending its analytical capabilities. TruTools offers advanced preprocessing of spectra, qualitative discrimination, quantitative component analysis, and a platform to deploy customized chemometric models including PCA, PLS, and PLSDA on board the handheld device.

Representative spectra of several protein products and a placebo display unique and reproducible Raman features, however, due to the minor structure modifications between proteins, the differences between the spectra can be relatively small (figures 3 and 4).

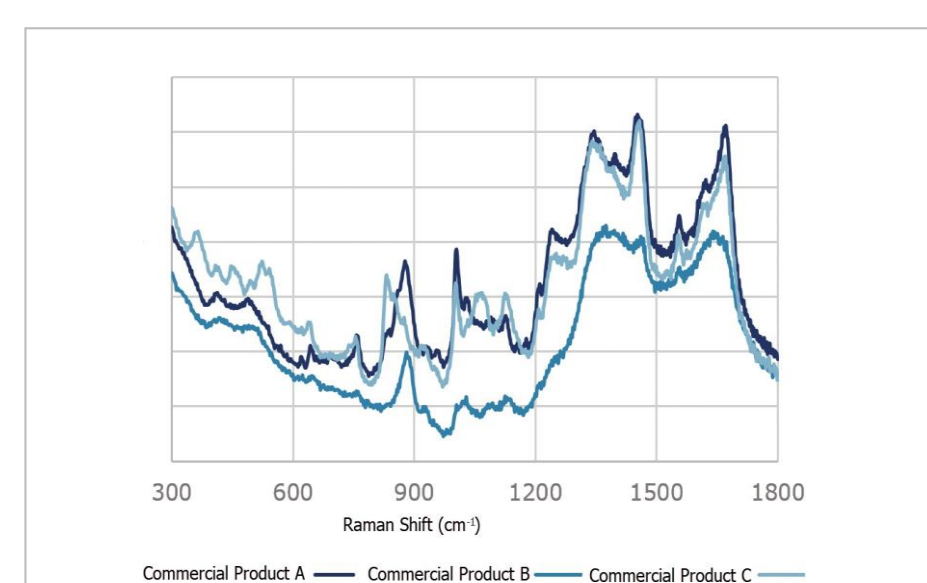


Figure 3. Raman spectra collected from TruScan RM of 3 commercial products

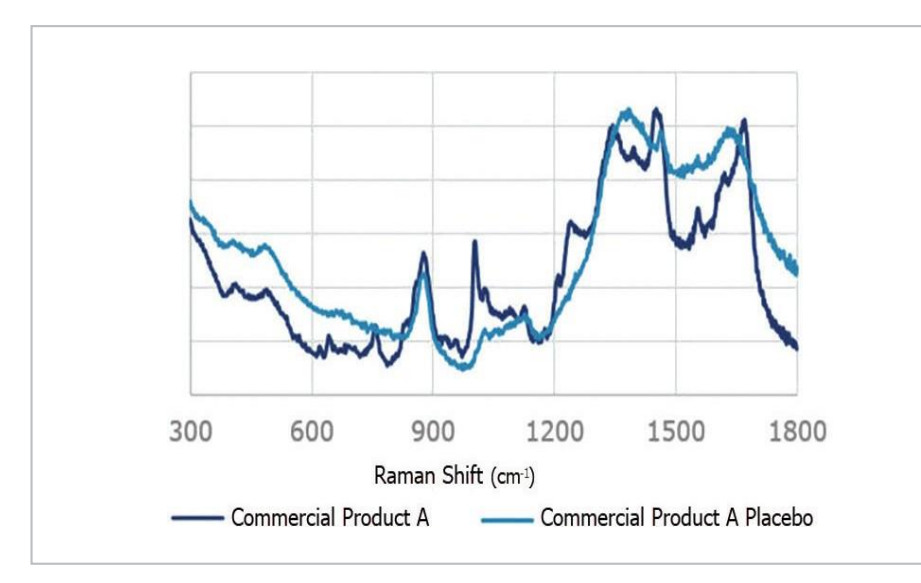


Figure 4. Raman spectra collected from TruScan RM of a commercial product and its placebo

TruTools chemometrics software has the capability to differentiate the other 5 similar protein products. Principal Component Analysis (PCA) and Partial Least-Squares Discriminant Analysis (PLSDA) were used to build models and the results showed clear separation between the various protein products as well as protein products and their placebos.

For both commercial products A and B, a PCA model alone delivered good separation (figure 5).

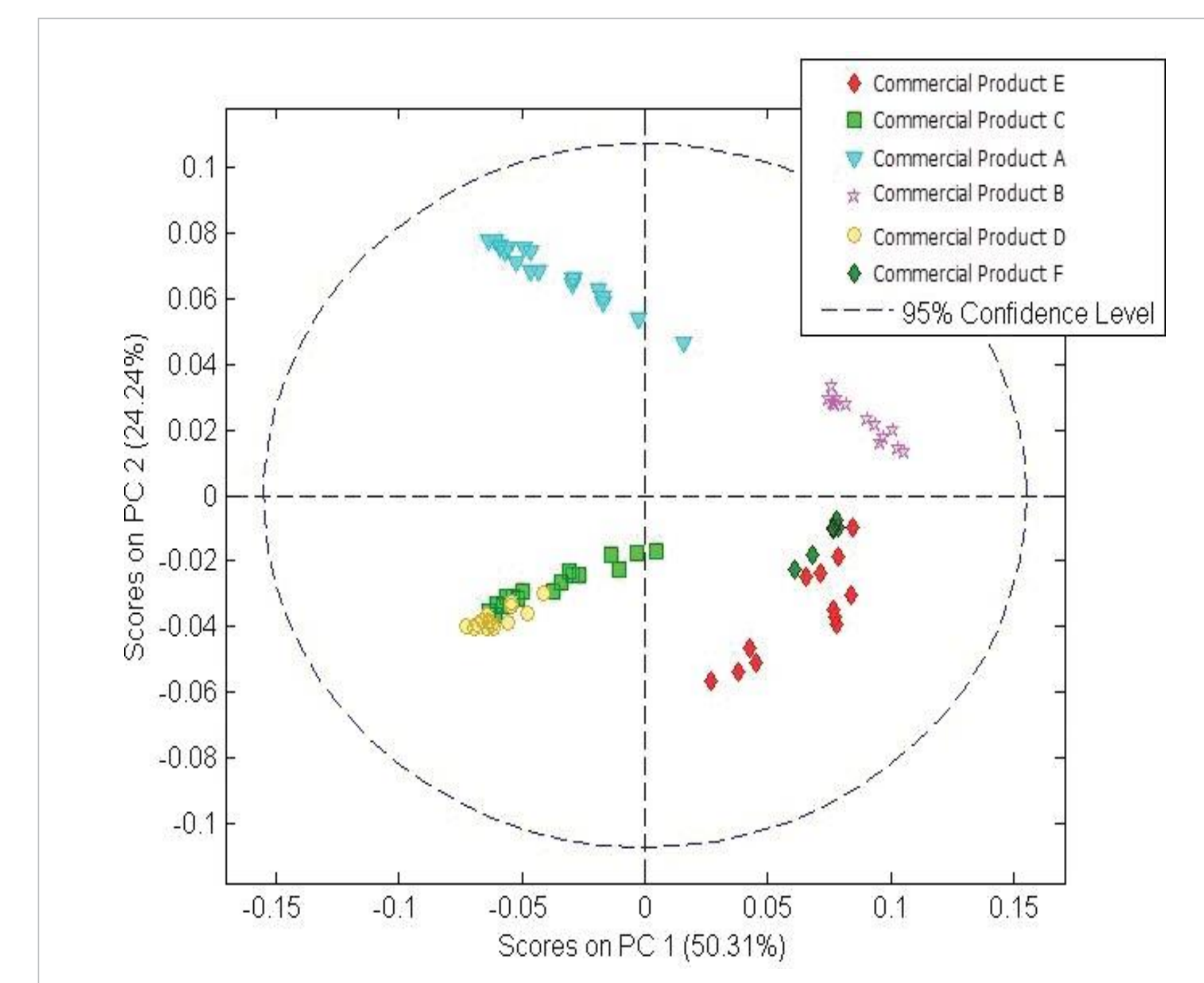


Figure 5. PCA model shows satisfactory separation for Commercial Products A and B

For the 4 remaining protein products a PLSDA model was developed as an extension of the PCA results for additional separation of the 4 classes (figure 6). In this case Commercial Product E and Commercial Product F, which were close neighbors in the PCA plot, are now definitively separated as different classes. Remaining models were then deployed in similar fashion to the TruScan RM analyzer and run as TruTools methods with good initial results.

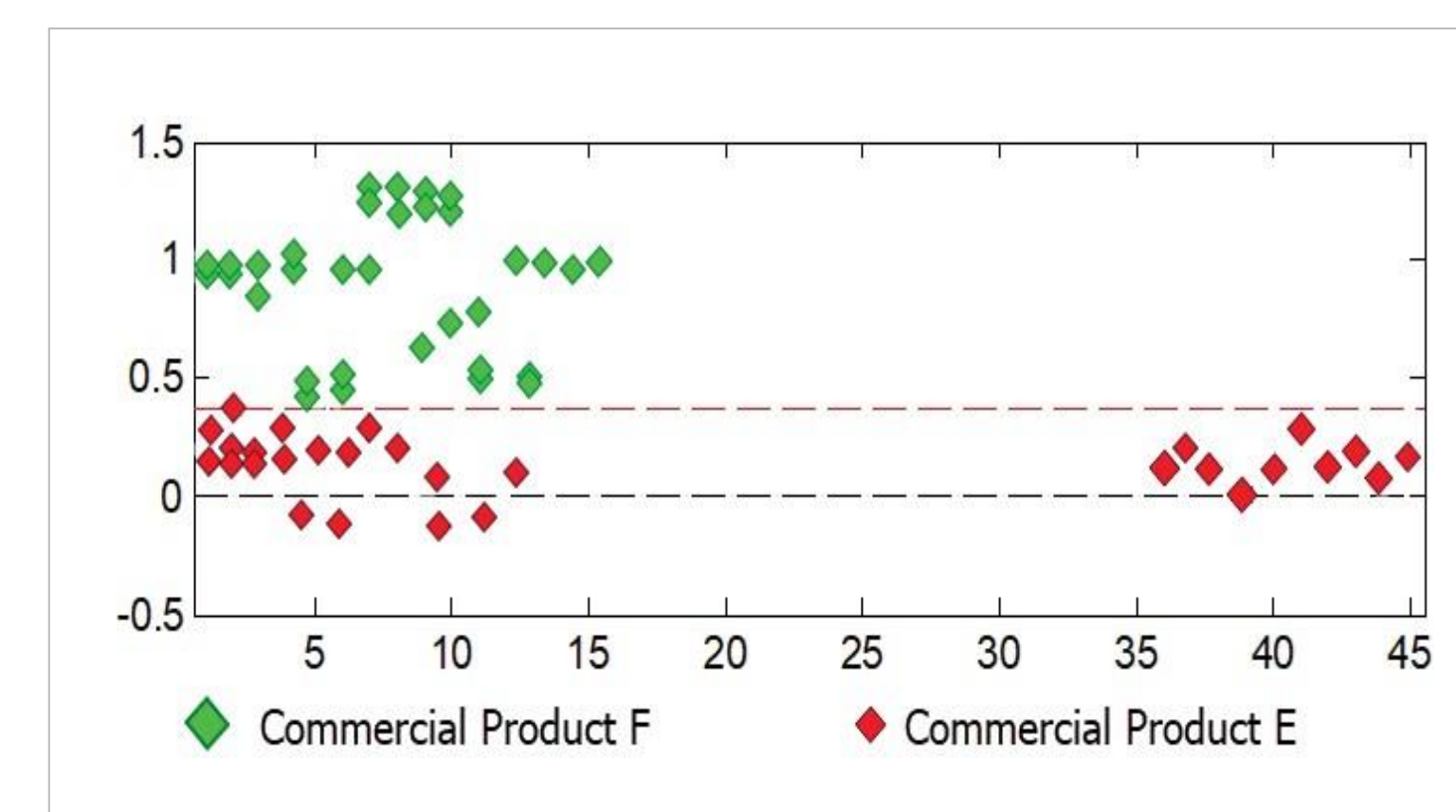


Figure 6. PLSDA model used to separate different classes



## CONCLUSIONS

In pharmaceutical manufacturing of chemical compounds, handheld Raman analyzers are widely accepted for material verification of excipients and APIs. For many years the pharmaceutical industry has used TruScan RM's embedded multivariate residual analysis decision engine to identify most raw materials. Moreover, TruScan RM's characteristics of non-contact, nondestructive testing, little or no sample preparation, high molecular specificity, and the ability to measure aqueous solutions make it suitable for larger molecule biologics testing. With the addition of TruTools on-board chemometrics, complex biological molecules can be discriminated. This can dramatically reduce the amount of time, technical resources, and expense required to conduct protein product ID tests.

It is noted that this feasibility study will be complemented by further validation of the robustness of the models to correctly predict the identity of the materials under evaluation by testing across additional commercial product batches and ranges of acceptable concentration. The ability to produce ID results on the order of a few minutes, versus days, will continue to drive the development and validation efforts forward.

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