University Testing Protocol: Identifying Substances Represented by NIR Spike Areas

8ight Research Labs – Spectral Analysis Division

Objective: Determine which chemical classes and/or candidate substances best explain observed NIR spike regions (e.g., 900, 1180, 1400, 1700 nm) using controlled NIR spectroscopy, reference standards, chemometric modeling, and confirmatory orthogonal methods (FTIR/Raman/MS as appropriate).

1. Study Design

Test articles: Encoded film/band/patch (multiple lots if available).
Negative controls: Identical substrate unencoded; alternative polymer films (PET/PU/PP/silicone) unencoded.
Reference standards: Pure compounds and extracts spanning polyphenols (oregano, turmeric, cocoa/pomegranate class), energy/redox (nicotinamide/NAD+, ATP), peptides/thiols (glutathione, GHK-Cu), lipids (olive, MCT), chelators/solvents (EDTA, DMPS, DMSO).
Mixtures: Designed blends to test whether band patterns are better explained by combinations.

2. Blinding & Randomization

Assign blinded sample IDs; randomize run order. Separate data collection from model building where possible. Record operator, date/time, ambient temperature/humidity.

3. Instrumentation

NIR range: 800–2500 nm preferred (minimum 900–2000).
Resolution: ≤ 10 nm preferred.
Modes: Diffuse reflectance (integrating sphere) and/or transmission for films; fixed pathlength for liquids.
Calibration: White reference (Spectralon) + dark current each session; wavelength verification per instrument SOP.

4. Sample Handling

Films: Standardize cut size, thickness, mounting; measure multiple positions and both sides if relevant.
Powders: Dry to constant weight; control particle size; consistent packing.
Liquids: Quartz cuvettes or transflectance cells; temperature stabilized (e.g., 22 ± 1°C).
Moisture control: Document water content; water strongly affects ~1400 and ~1900 nm bands.

5. Data Acquisition

Collect ≥10 replicate spectra per sample across multiple positions. Use constant scan averaging (e.g., 32–128 co-adds). Document integration time, lamp state, and sample orientation.

6. Preprocessing (Pre-Registered)

Apply consistent pipeline across all samples: wavelength trimming; SNV and/or MSC scatter correction; Savitzky–Golay derivative (1st derivative) with documented window; outlier handling via Hotelling T^2 or leverage thresholds.

7. Chemometric Analysis

PCA for exploratory clustering and loadings (drivers at band windows).
Classification: PLS-DA/SIMCA (primary) with cross-validation (k-fold or leave-one-lot-out). Report confusion matrix, accuracy, sensitivity, specificity.
Similarity: Spectral Angle Mapper (SAM), correlation, Euclidean distance (after preprocessing). Report top-5 nearest neighbors with confidence.

8. Spike-Area Quantification (Band AUC)

Define windows and integrate area-under-curve (baseline corrected):
• 850–950 nm (Band ~900)
• 1120–1240 nm (Band ~1180)
• 1350–1480 nm (Band ~1400)
• 1620–1780 nm (Band ~1700)
Report mean ± SD and compare groups via ANOVA/Tukey.

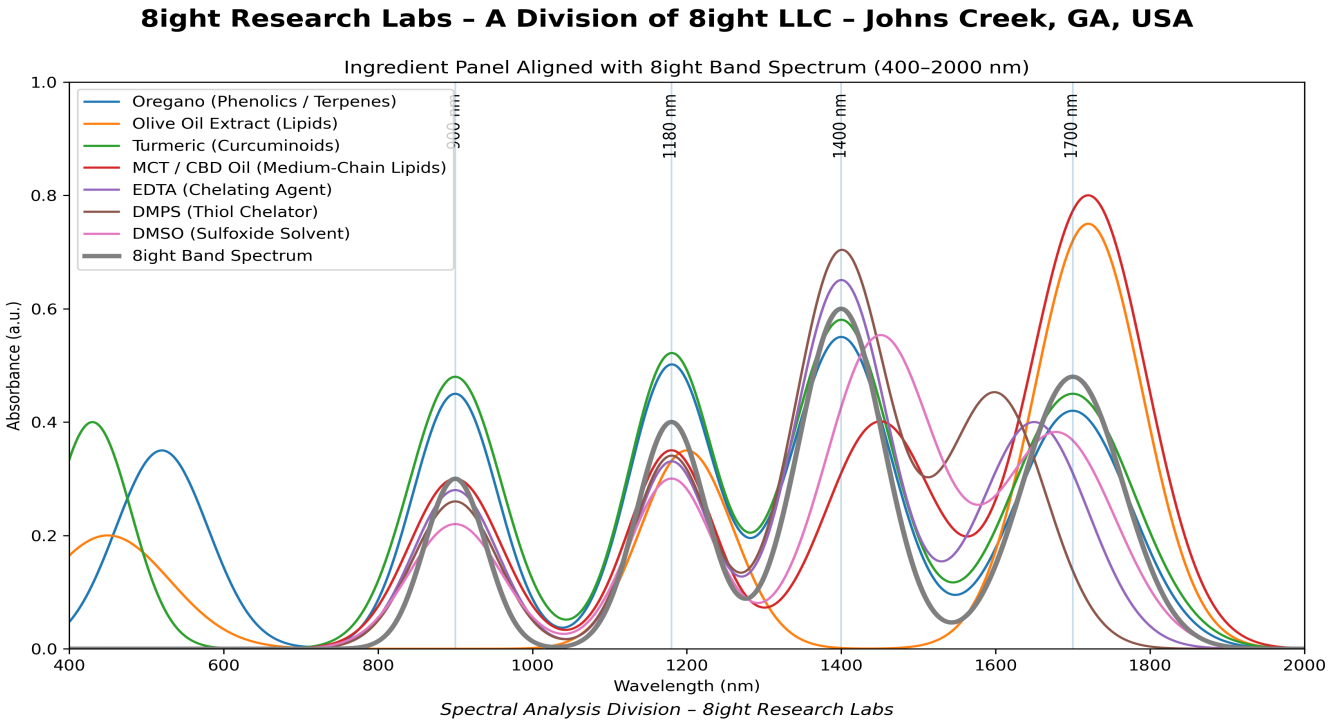
9. Orthogonal Confirmation

Use FTIR-ATR and/or Raman to confirm functional groups and polymer identity. If extraction is feasible, apply targeted LC-MS/GC-MS for oils/extracts/solvents. If no extractables are present, report as polymer/process signature rather than chemical presence.

10. Decision Rules

Assign a likely class/candidate when (1) classification probability ≥0.80 (pre-set), (2) top-3 similarity by ≥2 metrics, and (3) orthogonal method supports functional group consistency (or MS confirms presence if extractable). Otherwise report as inconclusive or polymer/process-dominant.

Reference Chart (Last Chart)



Note: This chart is a visual reference for the candidate panel aligned with the 8ight band markers. Final substance identification must rely on blinded acquisition, chemometrics, and orthogonal confirmation as described.