



Evaluation Ground Aromatic Medicinal Herb Samples Using ASD LabSpec® 5000 with Muglight attachment

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Introduction:

Samples of three ground herb types were received for model development on the ASD LabSpec NIR system. 27 Mint, 32 Greek Oregano and 7 Syrian Oregano.

All three sample types had primary chemistry data for Rosmarinic acid, Oleanolic acid, Ursolic acid and Total triterpenes, which is a combination of Oleanolic and Ursolic acids.

The LabSpec 5000 collects spectra from 350 to 2500nm with an increment of 1nm. This rugged portable instrumentation allows for a variety of sampling accessories to be used, including ASD's Muglight attachment.



Experimental:

The ground samples were placed into the muglight cuvette and reflectance spectra for all three sample types were collected for using ASD's LabSpec 5000 spectrometer system with the Muglight high intensity source probe. Spectra were collected using a 100 scan average with two replications per sample. This number of scans corresponds to a collection time of 10 seconds.

Spectra were then imported into the UNSCRAMBLER version 9.7, CAMO, Woodbridge, NJ for evaluation and modeling.

Duplicate spectra were then averaged and reference data was matched with the spectral data. Reference data used for this experiment are in Appendix A.

Spectra were converted from Reflectance to Absorbance ($\text{Log } 1/R$) for model development.

PLS1 models were developed for each sample type as well as for all sample types in the same calibration.

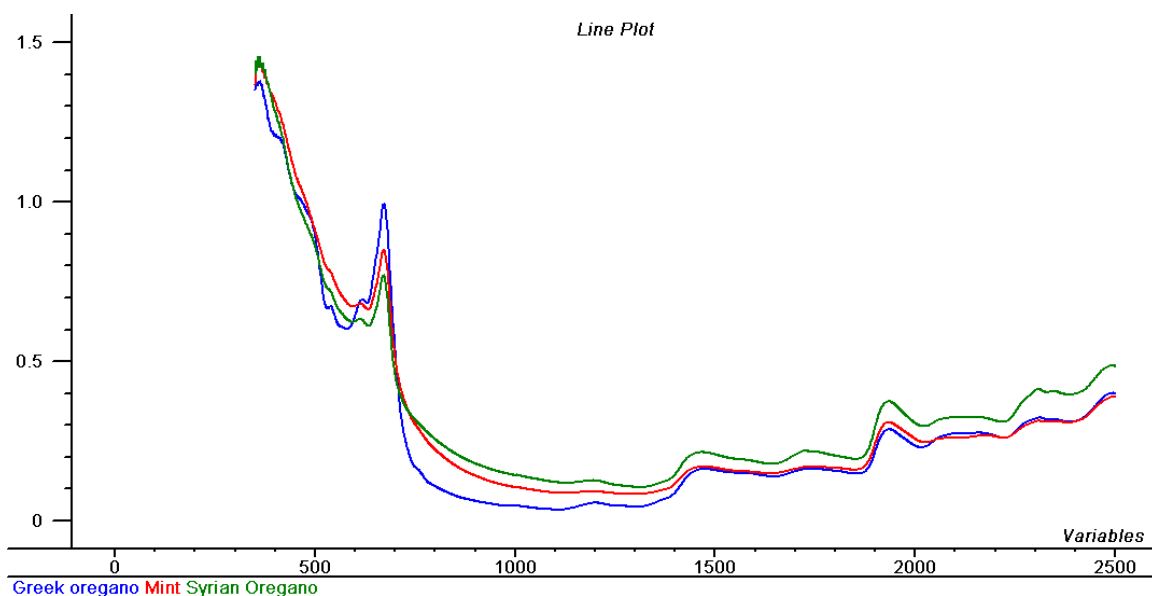
Both full cross-validation and true test set validations were used for calibration model validation. Samples were randomly assigned to either calibration or validation sets for creation of the true test set, 50 samples were used for the calibration set and 16 for the validation set. Test set validation was used only for the calibrations developed with all samples due to the limited number of samples available for this test.

Results:

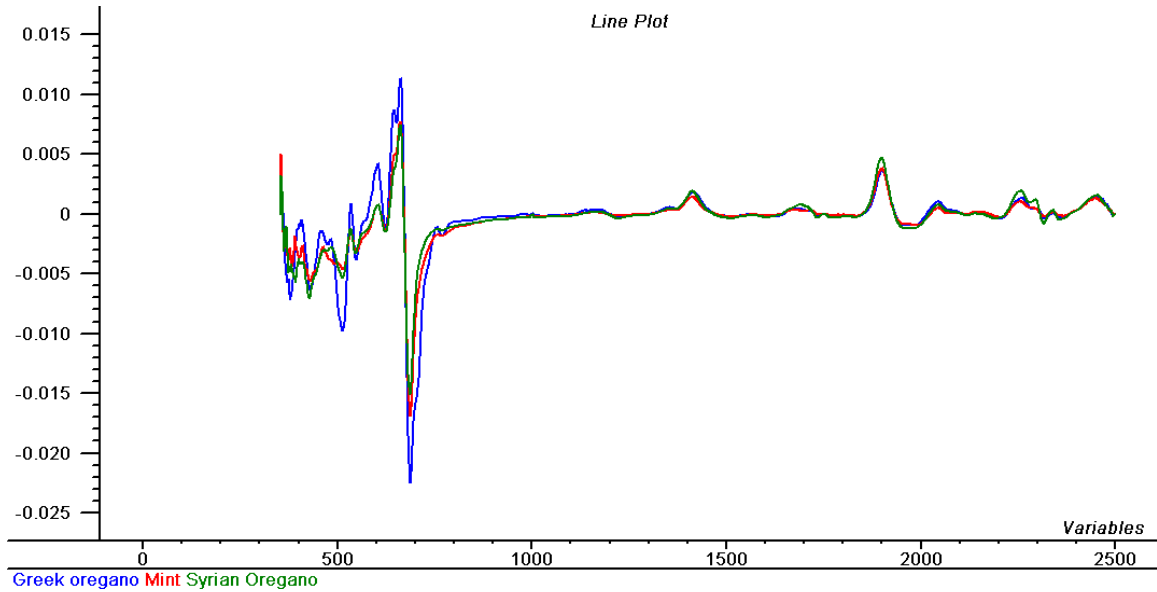
Savitzky-Golay 1st derivative with 11 point second order smoothing was used as a pretreatment in all PLS1 models presented for this study. Models created with SG1 pretreatment outperformed models created without this pretreatment. For clarity purpose only data from models with SG1 are presented in this report.

The two plots below show the difference in absorbance spectra between the three sample types.

Absorbance spectral comparison

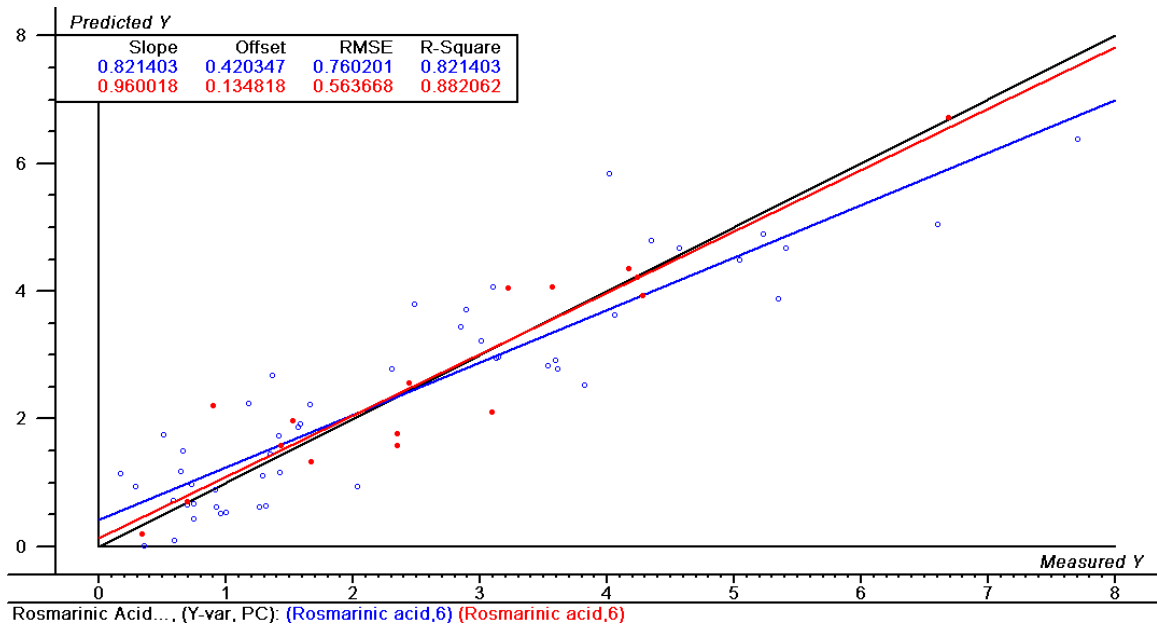


1st derivative Savitzky-Golay 11 pt 2nd order smoothed spectral comparison



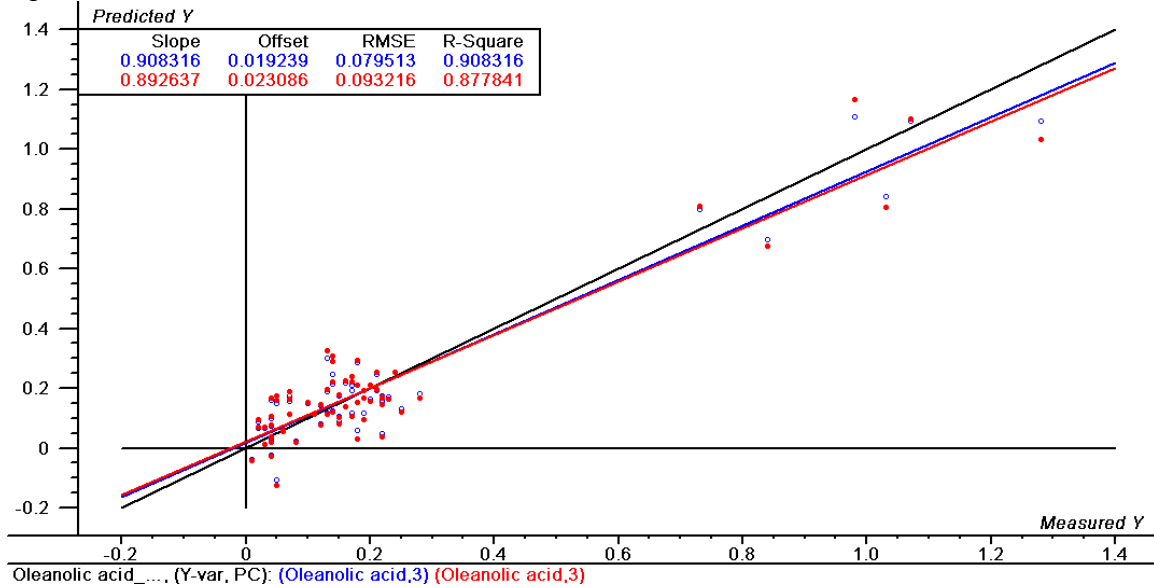
Rosmarinic Acid

The best model was obtained using the 50 sample calibration set with 16 sample true test set. Model used 6 factors (principle components), the calibration had a Root Mean Square Error of calibration (RMSEC) of 0.76 with RSQ of 0.82. The validation set had RMSEP of 0.56 and RSQ of 0.88.

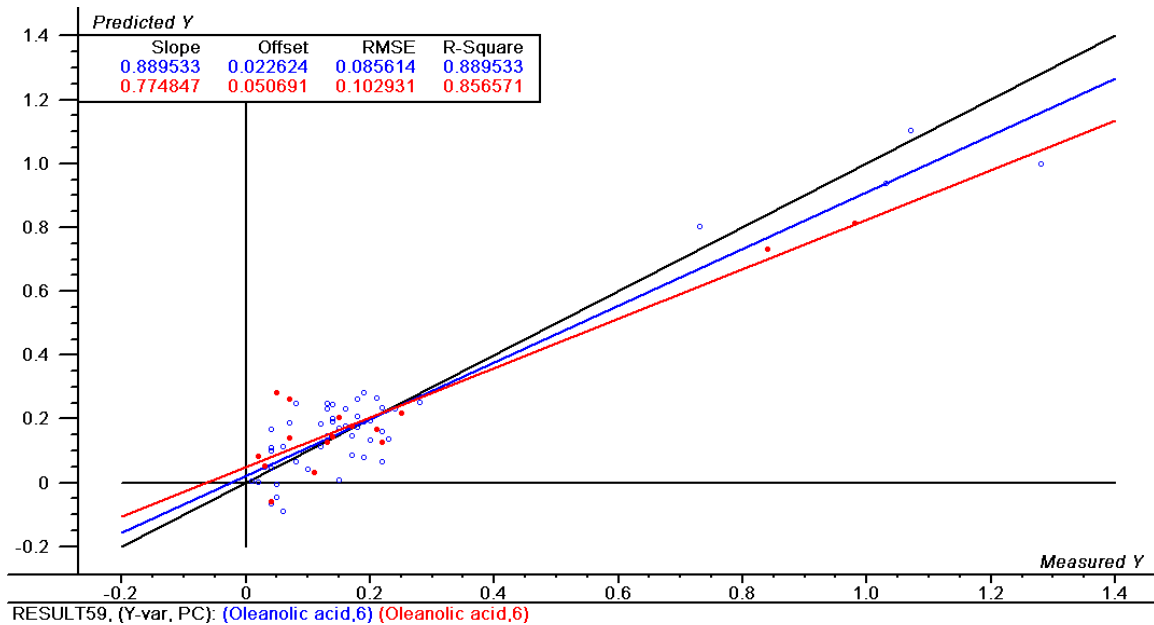


Oleanolic Acid

Since few of the total set included a wide range of Oleanolic acid, the best model results were from a calibration created with all 66 samples together. One outlier was removed and wavelengths were reduced to create a model with 3 factors. The RMSEC was 0.08 and RSQ was 0.91. Full cross validation was used in place of a true test set for this equation. RMSEP was 0.09 and RSQ was 0.88.

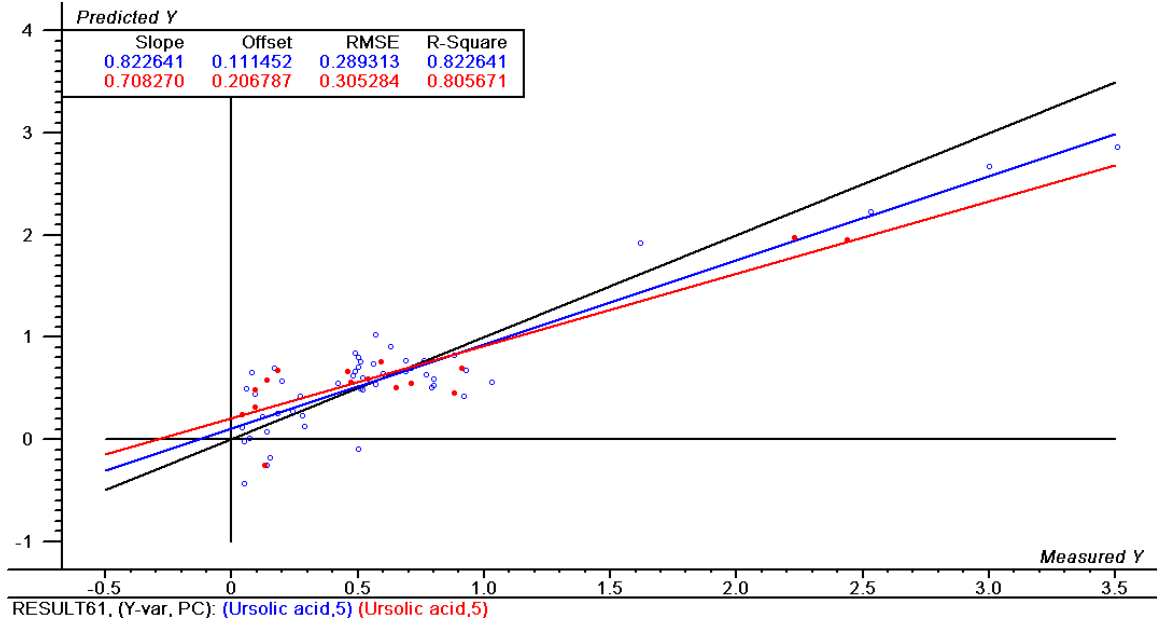


The calibration model produced using the 50 sample calibration and 16 sample test set yielded a 6 factor calibration with an RMSEC of 0.09 and RSQ of 0.89. RMSEP of the true test set was 0.10 with an RSQ of 0.86.



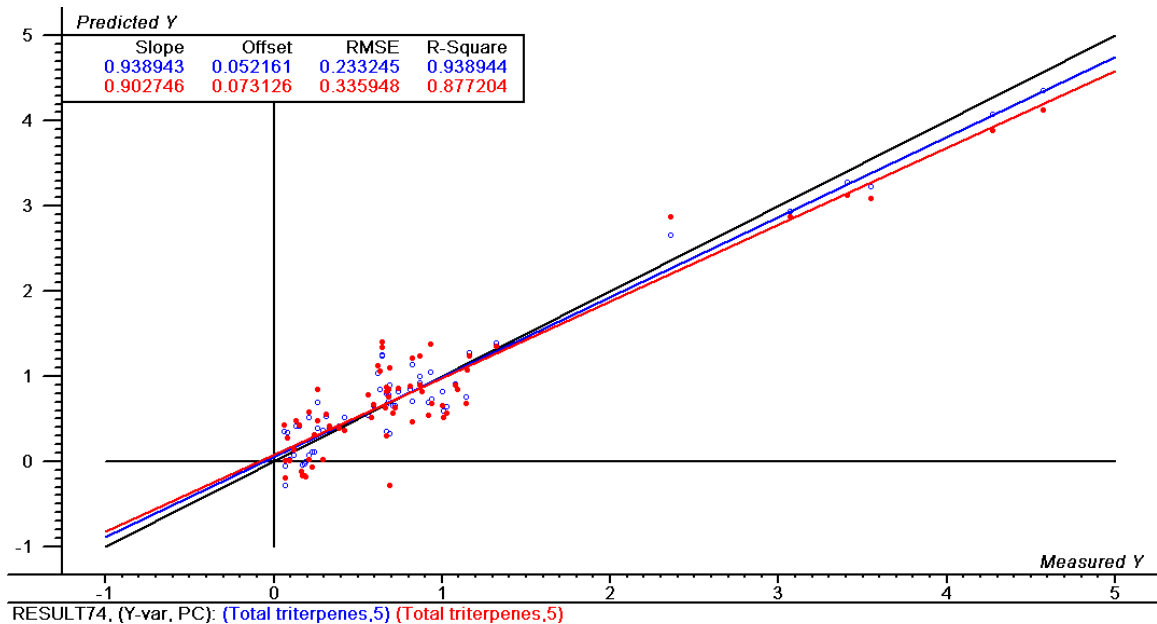
Ursolic Acid

Calibration using 50 sample cal set and 16 sample test set produced a 5 factor calibration with RMSEC of 0.29 and RSQ of 0.82. RMSEP of the true test set was 0.31 with RSQ of 0.81.



Total Triterpenes

As with Oleanolic acid, the best model results were from a calibration created with all 66 samples together. One outlier was removed and wavelengths were reduced to create a model with 5 factors. The RMSEC was 0.23 and RSQ was 0.94. Full cross validation was used in place of a true test set for this equation. RMSEP was 0.34 and RSQ was 0.88.



Calibration Summary

	Sample	n	factors	RMSEC	Cal RSQ	Validation type	RMSEP	Validation RSQ	Notes
Rosmarinic Acid	Greek Oregano	32	3	0.65	0.83	Full cross	0.82	0.75	
Rosmarinic Acid	All types	65	4	0.66	0.84	Full cross	0.75	0.80	
Rosmarinic Acid	All types	50	6	0.76	0.82	Test set (16)	0.56	0.88	
Oleanolic Acid	Syrian Oregano	7	2	0.10	0.92	Full cross	0.21	0.74	
Oleanolic Acid	All types	65	3	0.08	0.91	Full cross	0.09	0.88	
Oleanolic Acid	All types	50	6	0.09	0.88	Test set (16)	0.13	0.76	
Ursolic Acid	Syrian Oregano	7	2	0.29	0.92	Full cross	0.69	0.67	
Ursolic Acid	All types	65	7	0.16	0.94	Full cross	0.22	0.9	
Ursolic Acid	All types	50	5	0.29	0.82	Test set (16)	0.31	0.81	
Total Triterpenes	Syrian Oregano	7	2	0.36	0.93	Full cross	0.88	0.69	
Total Triterpenes	All types	65	5	0.37	0.85	Full cross	0.42	0.81	
Total Triterpenes	All types	50	6	0.29	0.9	Test set (16)	0.37	0.74	

Conclusion:

Calibration for the test constituents appears to be feasible; but samples with a wider constituent range are needed to produce a stable and robust calibration. Although differing sample types were combined into a single calibration model, this is not ideal with a relatively small number of calibration samples. However, cross validation and true test set results confirmed the predictive ability for combining these three sample types into a single calibration.

Following expansion of the constituent range, the calibration may be useful for rapid screening of these aromatic medicinal herbs.