

**SDSU  
Seed Testing  
Laboratory**  
**Varietal Verification or  
Identification using High  
Performance Liquid  
Chromatography (HPLC)**



**SOUTH DAKOTA  
STATE UNIVERSITY**

Member  
Association of Official Seed Analysts  
Society of Commercial Seed Technologists

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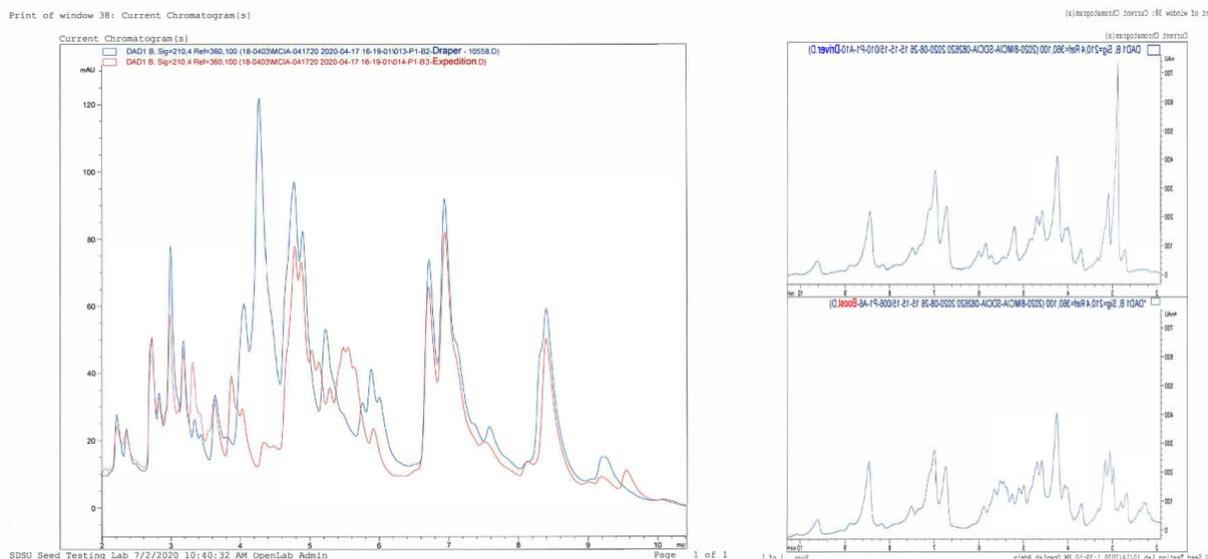
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<https://www.sdstate.edu/agronomy-horticulture-and-plant-science/sdsu-seed-testing-lab>

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## High Performance Liquid Chromatography (HPLC)

HPLC is a chemical separation technique that can separate alcohol-soluble proteins and other compounds. The instrument provides a “fingerprint pattern” for each variety of seed. A known check sample of the variety in question is processed and overlaid with the client’s sample for comparison and confirmation of the variety.

When cereal proteins are separated, the resulting protein peak pattern is used as a “fingerprint” for that particular variety. Using this technique for varietal verification/ID is fast and economical. Crop Improvement Associations use this technique for variety identification, but it has also become popular among seed companies, seedsmen, and farmers for varietal verification/ID of purchased seed (non-certified samples). There may be a situation where the producer isn’t 100% sure which bin has the winter wheat vs. the spring wheat seed - this can be a very costly mistake but is preventable with an HPLC test.



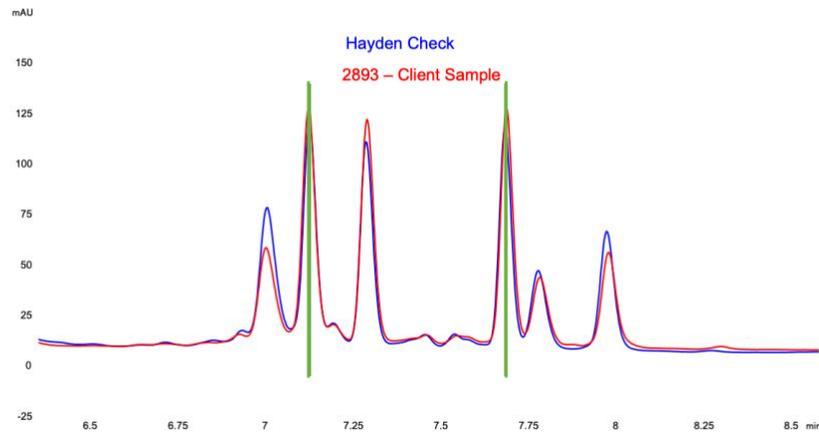
*Above left are overlain chromatograms of Draper (in blue) and Expedition (in red) HRW wheat. To the right are the chromatograms of Driver and Boost HRS wheat shown separated.*

The SDSU Seed Testing Lab currently tests wheat, oats, rye, barley, and triticale. Potentially any crop may be tested if a protocol can be developed – inquire as to possibilities. Samples submitted are assumed to be from a pure seed lot (single variety) and a bulk test (100 seeds) is performed. Single seeds or seed heads can also be tested. Plant breeders, foundation seed programs, and field inspectors often submit samples to check suspected off types seen in the field.

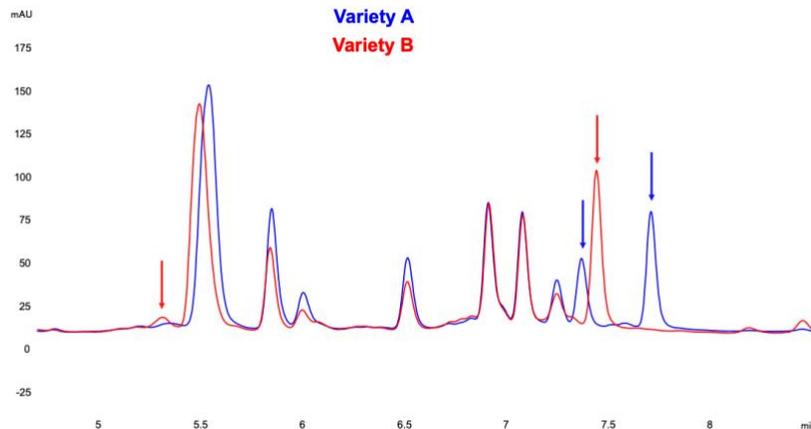
Varieties in a mixture can only be determined by single seed (SS) analysis. Such tests are more expensive due to the increased number of samples run versus one bulk test.

## How to use and read an HPLC Chromatogram

HPLC testing at SDSU utilizes the alcohol soluble proteins in a seed to obtain a “fingerprint” pattern which is unique to each variety. We then compare it to a check sample (known variety) to verify or identify the variety of the sample submitted. Below is a simplified explanation of a Hayden known check (in blue) vs. a client oat sample (in red) submitted as Hayden for verification. The two vertical green lines are used to help align peaks for evaluation.



The below chromatogram shows two oat varieties overlaid with some of the differences (protein peaks) between them marked with red and blue arrows. Wheat varieties have similar patterns but with many more protein peaks.



## Fees for HPLC

### **Bulk Seed Sample (Wheat/Oats/Rye/Triticale): \$60/sample + \$60/check**

Normally 100 pure seed units are randomly selected from the submitted sample and ground up. A portion of the flour is used for protein extraction. A check sample of the suspected variety(ies) is also extracted for comparison.



### **Single Seed (SS) (Wheat/Oats/Rye/Triticale): \$60/SS + \$60/bulk check**

Single seeds are individually ground and extracted, and a bulk sample of the suspected variety(ies) is also prepared for comparison.

**Barley, Rye & Triticale** – most varieties we have run can be distinguished from one another, but before sending, contact the lab for further information. Pricing is the same as above.

Other crops currently under research for HPLC analysis:

1. Rice
2. Field Pea
3. Chickpea

For information contact Dr. Brent Turnipseed in the SDSU Seed Testing Lab at 605-688-4590, or by email at [brent.turnipseed@sdstate.edu](mailto:brent.turnipseed@sdstate.edu).