EXPLORING PROTEIN PROFILES OF GRAINS BY HPLC

INTRODUCTION

HPLC is used at the South Dakota State University (SDSU) Seed Testing Laboratory to verify the identity of different seeds/grains in order to prevent mislabeling or fraud. The cereal proteins are extracted using ethanol/water and then they are analyzed by HPLC along with a reference seed sample. The peak pattern of the proteins is compared to confirm the identity of the seeds in question. All seeds have a unique cereal protein pattern. HALO 1000 Å Diphenyl provides excellent peak shape and resolution for the separation of cereal proteins. This column has mostly been used for monoclonal antibodies, biosimilars, and fusion proteins, but it is an excellent choice for any application that involves large molecules, such as this one. The combination of unique selectivity and high efficiency for even the largest molecules is effective for evaluating complex mixtures of seed proteins.

EXPERIMENTAL CONDITIONS:

Columns: as indicated, 4.6 x 150 mm

Mobile Phase A: Water/0.1% TFA

Mobile Phase B: Acetonitrile/0.1% TFA

Gradient:

Time	%B
0.00	25
1.25	35
10.75	50
11.00	95
14.00	95
14.10	25
18.00	25

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detection: Diode Array, 210 nm, 220 nm, 280 nm

Injection Volume: 6 µL

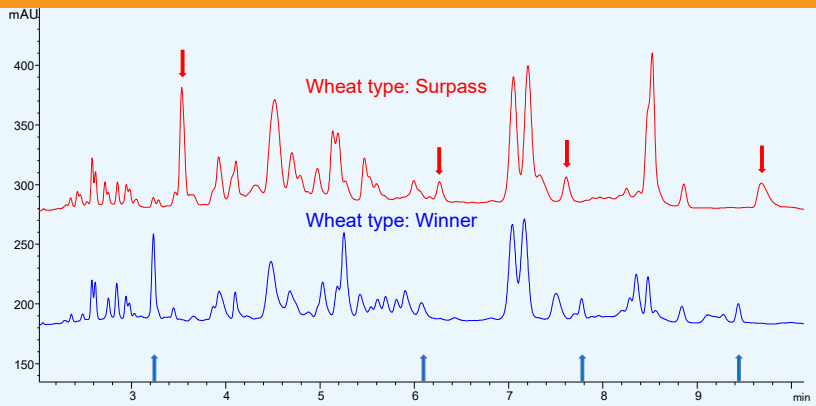
Sample Solvent: ethanol/water

Data Rate: 5 Hz

VARIETIES OF SEEDS TESTED

Figure 1 shows two different varieties of wheat: Surpass and Winner separated on the HALO 1000 Å Diphenyl column. High protein levels that exhibit a broad range of hydrophobicity are evident for the wheat varieties of grains. The high abundance of peaks and complex pattern can make identifying the wheat type challenging. Still, a stable, high-resolution separation, such as that provided by this column, allows the laboratory to identify peaks that are unique to each variety. These peaks are noted in Figure 1.

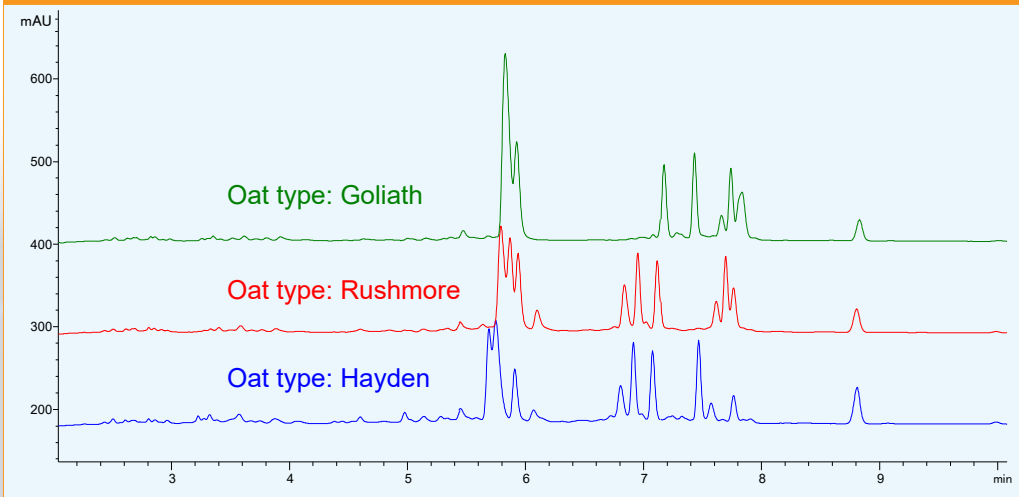
Figure 1. Cereal proteins extracted from Surpass and Winner wheat varieties separated using a HALO 1000 Å Diphenyl column.



Courtesy: South Dakota State University Seed Testing Laboratory and Merlin K. L. Bicking, Ph. D. (ACCTA).

The next grain example features three different types of oats: Goliath, Rushmore, and Hayden, and characteristic patterns are observed with each oat variety on the HALO 1000 Å Diphenyl column, making identification less challenging. See Figure 2.

Figure 2. Comparison of cereal proteins extracted from three different varieties of oats separated using a HALO 1000 Å Diphenyl column.



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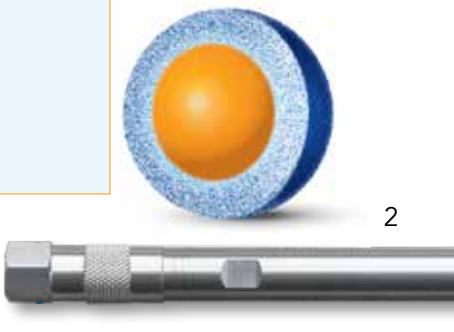
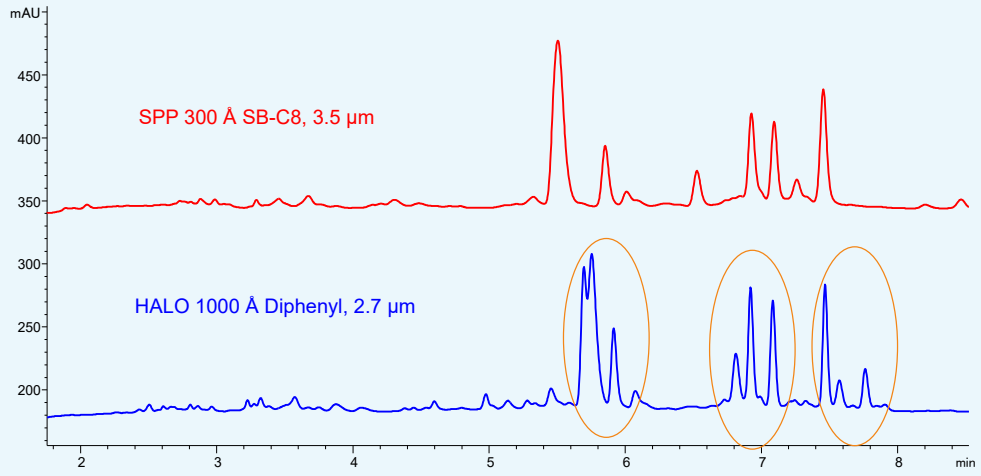


The results for the Hayden oat type were compared using a HALO 1000 Å Diphenyl, 2.7 µm to a SPP 300 Å SB-C8, 3.5 µm column in Figure 3. Improved selectivity and sharper peak shapes are observed on the HALO 1000 Å Diphenyl column (circled groupings) due to the larger pores that enable improved access to the stationary phase, which largely resides in the pores. Comparing the first major peak, the HALO Diphenyl column is beginning to resolve an additional peak while those peaks are coeluted on the 300 Å column. Additional phase screening can be done in order to maximize resolution. For the purposes of these tests, the gradient conditions were not optimized.

Figure 3. Comparison of cereal proteins extracted from Hayden oats showing improved peak shape and selectivity with the HALO 1000 Å Diphenyl column.



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The last example in Figure 4 shows the proteins in three different varieties of rice separated using the HALO 1000 Å Diphenyl column. Compared to other grains, less protein is observed (lower number of peaks and reduced intensity). These results are consistent with the fact that rice has less protein by weight than the other grains. However, the high resolution and unique selectivity of this column still allow the identification of unique peaks in each variety.

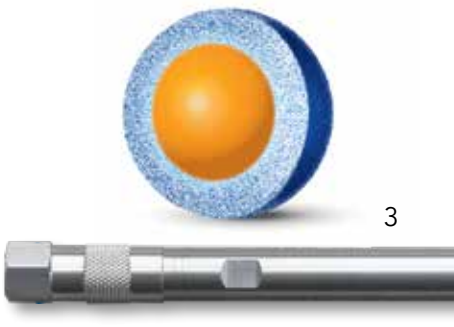
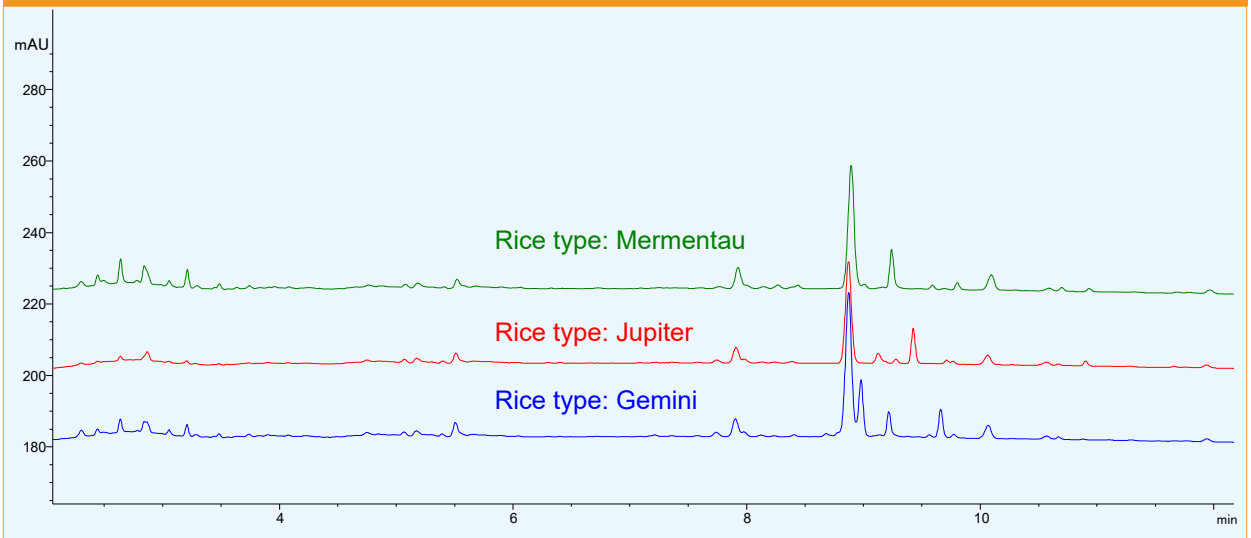


Figure 4. Comparison of cereal proteins extracted from three different rice varieties using the HALO 1000 Å Diphenyl column.



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CONCLUSION

The HALO 1000 Å Diphenyl column was utilized to investigate protein profiles from a variety of different grains for identification purposes. LC-MS analysis can further be used in order to have a better understanding of protein identification. This work is critical for seeds that have not been certified in order to prevent mistakes such as spring varieties being planted in the winter. These examples demonstrate that the HALO 1000 Å Diphenyl can be expanded for use in protein analysis other than biotherapeutics alone.