Waters
Lab Highlights
An Internal Communication of Applications and Techniques
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ANION EXCHANGE LC OF WHEAT PROTEINS

One of our customers was interested in fingerprinting wheat proteins (approx. mol. wt. 15,000-3,000,000; pI 5.0-8.0) by HPLC. He had previously explored a reversed-phase system and found that it was not possible to obtain reproducible chromatograms. To meet the customer's needs, an anion-exchange (DEAE) HPLC procedure was developed which resulted in excellent reproducibility.

A typical chromatogram of a salt (0.5 M sodium chloride) extract of wheat proteins is shown in Figure 1.

Prior to HPLC it was necessary to clarify the sample by filtration (Millex-SR; 0.5 µm). Proteins from clarified samples were resolved into two groups (Figure 1). The first group of proteins eluted within 4 mins. with 0.02 M Tris. Upon initiation of the salt gradient, several other proteins eluted between 11 and 17 mins. Similar results were also obtained with a second sample preparation. The chromatographic reproducibility of retention times of the major peaks is shown in Table 1.

FIGURE 1

Column: DEAE-5PW
(7.5 cm X 7.5 mm; i.d.)
Mobile Phase: A) 0.02 M Tris; pH 8.0
B) 0.02 M Tris/
0.5 M NaCl;pH 8.0
Gradient: 100% A for 5 min.
0% B to 100% B; 20 min.
Curve 6
Flow Rate: 1 ml/min.
Detector: 441, 280 nm

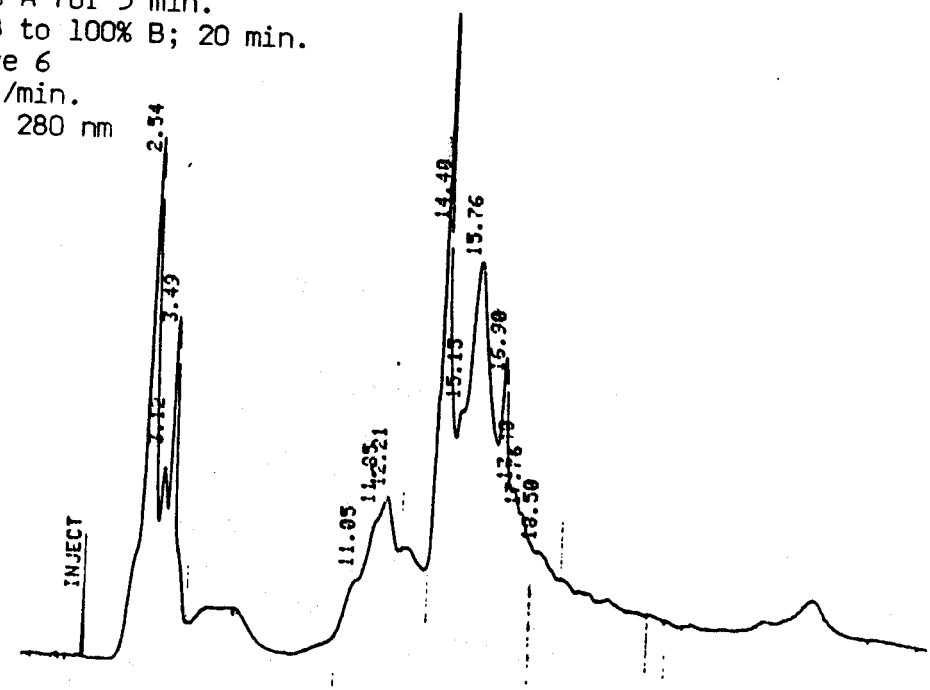

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TABLE 1

Injection No.	R_t of Major Peaks					Sample Prep No.
	1	2	3	4	5	
1	2.5	3.5	14.4	15.8	17.0	1
2	2.5	3.5	14.4	15.7	16.8	2
3	2.5	3.6	14.5	15.8	16.9	2
4	2.5	3.5	14.4	15.8	16.9	2
5	2.6	3.4	14.3	15.7	16.9	2
6	2.6	3.8	14.3	15.8	16.9	2