Meeting Nutrition Labeling Requirements

94-0355

☐ Waters™ columns and sample preparation products for nutrient analysis.

Table 1: Nutrients to be Listed on Full Label as in NLEA Proposals.

Item	Units
Calories	Per serving
Calories from total fat	Per serving
Total fat	Grams per serving
Saturated fat	Grams per serving
Cholesterol	Milligrams per serving
Total carbohydrate	Grams per serving
Complex carbohydrate	Grams per serving
Sugars	Grams per serving
Dietary fiber	Grams per serving
Protein	Grams per serving
Sodium	Milligrams per serving
Vitamin A	Percent RDI*
Vitamin C	Percent RDI
Calcium	Percent RDI
Iron	Percent RDI

* Recommended daily intake.

November 8, 1990 marked the beginning of the transition from mostly voluntary labeling of nutrition information to mandatory labeling. On that date, the U.S. Congress passed the Nutrition Labeling and Education Act of 1990 (NLEA). This law is intended to respond to consumer demands for meaningful ingredient and nutrition information. This past November, the United States Department of Agriculture (USDA) and Food and Drug Administration (FDA) proposed final regulations to meet the goals of the NLEA.

The Ramifications of the New Labeling Proposals

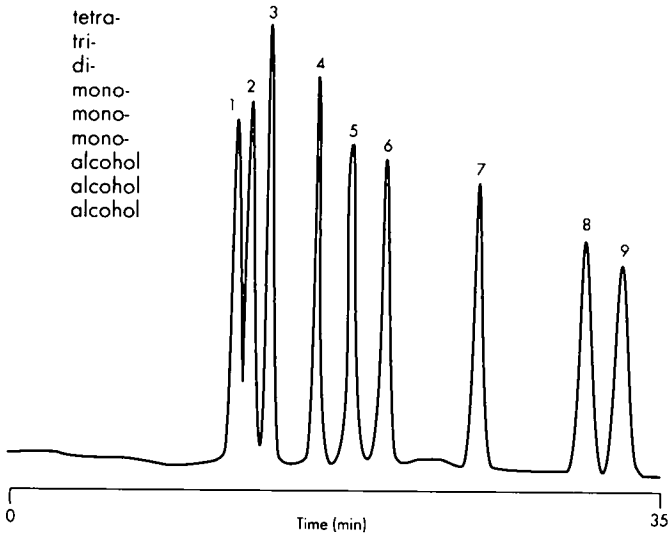
Upon adoption of the Food and Drug Administration's recently published labeling proposals, all processed foods will be required to carry nutrition information presented on a per serving basis. Table 1 lists the type of information food labels must carry under the new proposals. Food company analytical laboratories and commercial contract laboratories are expanding to meet the anticipated demand spawned by the new regulations. The proposed regulations will apply to foods imported into the U.S. as well.

Measuring the full range of nutrients in Table 1 will necessitate the use of sophisticated analytical techniques that go beyond proximate analysis. The most versatile technique is high performance liquid chromatography (HPLC). HPLC measures (1) simple sugars, (2) vitamins, (3) cholesterol, and (4) amino acids (as part of a protein quality assay). The FDA has indicated that HPLC will be the recommended technology for the compliance labeling of simple sugars. HPLC is also routinely used in laboratories today to analyze vitamins, including the fat soluble vitamins such as vitamin A as well as the B vitamins.

Figure 1: HPLC Separation of Mono-, Di-, Tri- and Tetrasaccharides and Sugar Alcohols

HPLC System: Waters™ Sugar Analysis System
Column: SP-1010 Precolumn, KS 801 + SP-0810 at 80°C
Detection: Waters 410 Differential Refractometer
Injection volume: 50 µL of 250 µg/mL standard

Specific Sugar	Class of Saccharide
1. Stachyose	tetra-
2. Raffinose	tri-
3. Sucrose	di-
4. Glucose	mono-
5. Galactose	mono-
6. Fructose	mono-
7. Mannitol	alcohol
8. Xylitol	alcohol
9. Sorbitol	alcohol

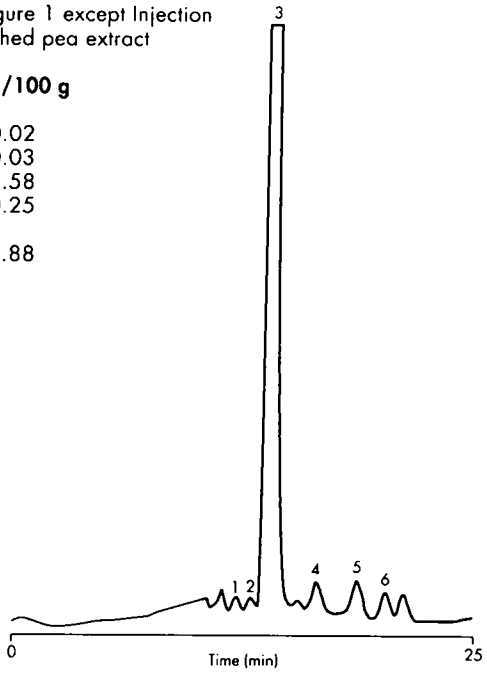


This separation of carbohydrate standards typifies the range required by the NLEA nutrition labeling proposals for simple sugars and sugar alcohols in foods. KS-801 and SP-0810 columns in series and a Waters 410 Differential Refractometer offer a simple way to measure these nutrients.

Figure 2: Sugar Labeling Analysis from Mashed Peas

Conditions: Same as Figure 1 except Injection volume = 100µL of mashed pea extract

Class of Saccharide	g/100 g
1. tetra-	0.02
2. tri-	0.03
3. di-	3.58
4 - 6. mono-	0.25
Total	3.88



Using the same HPLC system as in Figure 1, a sample of mashed peas was analyzed for its sugar content. Sep-Pak Plus C_{18} cartridges simplify sample preparation while helping to prolong column life.

Simple Sugar Analysis

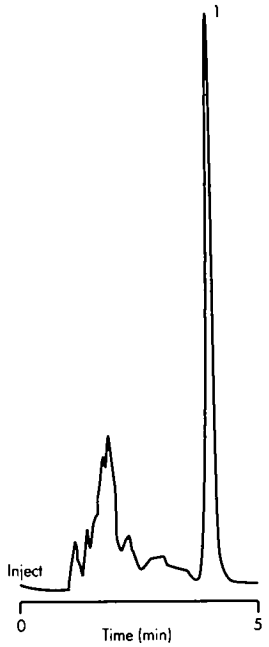
We offer a choice of sugar analysis columns and complete methodologies to meet the needs of particular food analyses.[2] For example, Waters™ Carbohydrate Analysis column is specified by the Association of Official Analytical Chemists (AOAC) for the separation of mono- and disaccharides from cereals and chocolate.[3] To quantify a broader variety of sugars, columns can be used in tandem to provide unique selectivity. Figure 1 is a separation of mono-, di-, tri-, and tetrasaccharides and sugar alcohols as analyzed with a KS-801 column and an SP-0810 column in series. These sugars are those proposed in the NLEA for labeling.

Another important aspect of sugar analysis is sample preparation. To cope with certain complex matrices, extraction of the analytes of interest and removal of unwanted components such as proteins, phenolics and acids may be necessary. These compounds can interfere with accurate quantitation of the sugars and shorten the life of a column. Waters Sep-Pak® cartridges are specified in official AOAC methods for analyzing sugars in foods[4] and have been cited in the literature numerous times for the analysis of sugars from foods and agricultural products.[5] Figure 2 shows how a Sep-Pak C_{18} cartridge was used to prepare a mashed pea sample for HPLC analysis. The sample was solubilized in water via sonication, filtered through filter paper, and extracted with a Sep-Pak C_{18} cartridge.

Figure 3: Vitamin A (as Retinol) Analysis in a Dairy Product

System: Waters™ LC Module 1
Column: Waters Nova-Pak® C_{18}, 3.9mm X 150mm
Injection volume: 25 µL of dairy product extract
Detection: UV

1. Retinol 4.3 µg/100 mL



Saponifying this food sample converted all ester forms of retinol to alcohol. Retinol can be easily resolved on a Nova-Pak C_{18} column and detected by UV. HPLC methods for vitamin A analysis are quickly being adopted as official methods because of their specificity and sensitivity.

Vitamin Analysis

Under the new nutrition labeling proposals, food labels will have to list vitamin A (defined as retinol and B-carotene) if it exists in processed foods at 2% or more of the recommended daily intake (RDI). A single Waters HPLC system can measure both nutrients with a Waters Nova-Pak® C_{18} column. An example of vitamin A analysis is shown in Figure 3.

Many labs rely on the specificity and sensitivity of HPLC over traditional spectroscopic procedures. Also, a recent joint FDA - Infant Formula Council collaborative study for the HPLC analysis of vitamin A from milk-based infant formula, yielded excellent results and was granted AOAC First Action approval.[6]

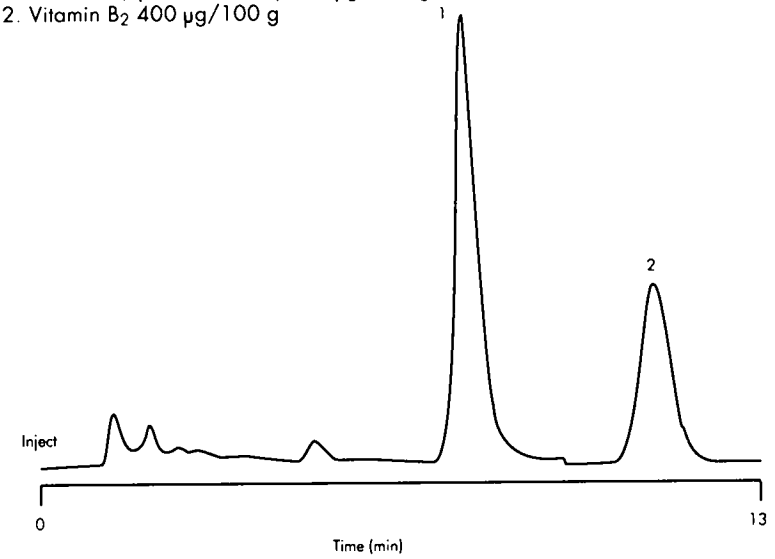
Some of the other vitamins will require labeling only if the food is claimed to be "fortified". Vitamins B_1 and B_2 fall into this category. Recent work by Waters applications chemists has focused on the analysis of these vitamins from a variety of sample matrices. Figure 4 depicts the simultaneous measurement of vitamins B. and B_2 from flour using Waters µBondapak™ C_{18} column. This application was highlighted in a previous issue of the *Waters Column* newsletter.[7]

In Figure 4, Sep-Pak Plus C_{18} cartridges were used to remove coextractives and excess reagents from the flour sample.

Figure 4: Vitamins B_1 and B_2 from Flour

System: Waters™ Isocratic HPLC System
Column: µBondapak™ C_{18}, 3.9mm X 300mm
Injection volume: 50 µL of flour extract
Detection: 470 Scanning Fluorescence

1. Vitamin B_1 (as thiochrome) 700 µg/100 g
2. Vitamin B_2 400 µg/100 g

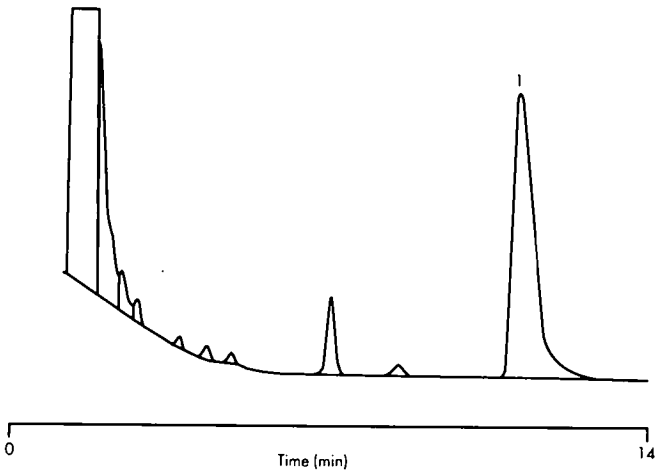


Vitamins B_1 and B_2 can be measured simultaneously by HPLC. The flour extract is passed across a Sep-Pak C_{18} cartridge prior to injection on to a µBondapak C_{18} column followed by fluorescence detection with a Waters 470 Scanning Fluorescence Detector.

Figure 5: Cholesterol in Turkey Extract

System: Waters™ LC Module 1
Column: Waters Nova-Pak® C_{18}, 3.9mm X 150mm
Detection: UV
Injection volume: 50 µL turkey extract

1. Cholesterol 10 mg/oz.



HPLC is an excellent alternative to wet chemical methods for cholesterol analysis. The system required is the same as that for vitamin A analysis when using UV detection. The chromatogram is courtesy of Syracuse Research Corporation, Syracuse, New York.

Cholesterol Analysis

Current methods of cholesterol analysis include titration, spectrophotometry and gas chromatography. HPLC is an excellent alternative noted for its specificity and ease-of-use since a derivatization step isn't required. Figure 5 depicts an HPLC cholesterol analysis from a turkey sample using Waters Nova-Pak C_{18} column. The separation was achieved in a non-aqueous reversed phase system and the cholesterol in the sample was detected by low wavelength UV absorbance. Following extraction and saponification, a Sep-Pak Plus C_{18} cartridge was used to remove coextractives and excess reagents.

An Array of Products for Nutrition Analysis

When it comes to HPLC nutrition analysis, no company has as many solutions as Millipore. For more information on the complete line of Waters HPLC products—instruments, data management systems, columns, and supplies—consult with your Millipore technical representative today.

Ordering Information

Item	P/N	Price
Nova-Pak C_{18}, 3.9mm X 150mm	86344	$295.00
µBondapak C_{18}, 3.9mm X 300mm	27324	365.00
SP-1010P Precolumn, 6mm x 50mm	34245	250.00
SP-0810 column, 1 x 10^3, Pb^{2+}, 8mm x 300mm	36954	700.00
KS-801 column, 1 x 10^3, 8mm x 300mm	34276	750.00
Sep-Pak Plus C_{18} Solid Phase Extraction Cartridges (Box of 50)	20515	105.00
LC Module 1	34551	
470 Scanning Fluorescence Detector	31300	
410 Differential Refractometer	70000	

References:
1. *Fed. Regist.*, 1991, 56 (229)
2. *Waters Column*, Winter 1991, pgs. 9-10, 14
3. *Official Methods of Analysis of the Association of Official Analytical Chemist, 15th Edition*, AOAC, Arlington, VA 1990
4. Ibid.
5. *Waters Sep-Pak Cartridge Applications Bibliography, Fifth Edition*, Millipore, Milford, MA, 1991
6. *The Referee, Association of Official Analytical Chemists*, 1992, 16 (1), pg. 4
7. *Waters Column*, Autumn 1991, pg. 10

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