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Validation of a size exclusion liquid chromatographic method for determination of methylcellulose and hydroxypropyl methylcellulose food gums as soluble dietary fiber in food and food product

Balasaheb Deshmukh [a,*], Robert G. Harfmann [a], Jerry Conklin [b], Maciej Turowski [b], Stephanie Lynch [b]

[a] *Analytical Sciences R&D, The Dow Chemical Company, 1897 Analytical Sciences, Midland, MI 48667, USA*
[b] *Water Soluble Polymers, The Dow Chemical Company, 1691 N. Swede Rd., Midland, MI 48674, USA*

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Abstract

A size exclusion liquid chromatographic method was developed for the determination of methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) as dietary fiber in food. These modified cellulose food gums are used in a wide variety of foods and physiologically behave as dietary fibers but are not determined using existing analytical dietary fiber methods. This article reports a single laboratory validation of a new method based on AOAC 991.43, and uses a liquid chromatograph with a refractive index detector. This new method was validated for foods containing 0.2–27% of MC and HPMC employing AOAC's Single Laboratory Validation protocol. Ten food samples of meat, bread, milk powder, potato and orange juice drink mix were studied. Precision of the new method, measured as total standard deviation (St), varied from 0.01 to 2 for foods containing 0.2–27% MC; and from 0.05 to 0.2 for foods containing 0.5–3.8% HPMC. Recovery varied from 76% to 85% for MC, and from 75% to 113% for HPMC. Use of a blank matrix proved successful in correcting for indigenous food fibers. The method demonstrated excellent linearity down to 0.03%. This provides the sensitivity required for food nutrition labeling purposes. These results show the method is suitable for determination of MC and HPMC in food and support further validation through a collaborative study.
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1. Introduction

Methylcellulose and hydroxypropyl methylcellulose are water-soluble fibers which have been used in food for decades to enhance the manufacturing process and improve food product qualities (Holownia, Chinnan, Erickson, & Mallikarjunan, 2000; Lovric, Boskovic, & Sablek, 1966; Schwitzguebl, 1990). These methylcellulose food gums (MFG) impart a range of desirable organoleptic qualities including enhanced 'mouth feel' which can improve taste properties of food (Barcenas et al., 2005; Ganz, 1973; Taylor Andrew, Hollowood Tracey, Davidson Jim, Cook David, & Linforth Rob, 2003; Virgallito, 2006). Additionally, these food fibers are known to resist digestion in the human gastrointestinal tract (Machle, Heyroth, & Witherup, 1944) and have been shown to provide physiological benefits associated with dietary fibers (Dressman et al., 1993; Maki et al., 1999, 2000; Reppas et al., 1993; Swidan et al., 1996; Tikhonova, Berdichevsky, & Paley, 1973; Topping, Oakenfull, Trimble, & Illman, 1988). As such, they meet physiological definitions of dietary fibers, such as

* Corresponding author. Tel.: +1 989 636 5507; fax: +1 989 636 6432.
E-mail address: bkdeshmukh@dow.com (B. Deshmukh).

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the definitions proposed by the Association of American Cereal Chemists, the Australia New Zealand Food Authority, and the National Academy of Sciences. These MC and HPMC fibers are also soluble in aqueous ethanol (Archer, 1992; Hess & Roland, 1986) which prevents them from being detected as soluble dietary fiber (SDF) using the widely accepted analytical enzymatic-gravimetric methods AOAC 985.29 and AOAC 991.43. The inability to detect ethanol-soluble dietary fibers by existing enzymatic-gravimetric procedures has prompted development of methods for specific dietary fibers such as polydextrose (AOAC, 2000), maltodextrin (AOAC, 2001), and resistant starch (AOAC, 2002).

The method described herein was validated for the determination of MFG as dietary fiber in foods. MC and HPMC were incorporated in five food matrices representing meat, dairy, grain, vegetables, and fruit at concentrations covering current and anticipated future food use. MFG in the subsequently dried and homogenized food samples ranged from 0.2% to 27%. The method was validated for methylcellulose and also for hydroxypropyl methylcellulose containing 27–30% methoxyl and 4–7.5% hydroxypropyl substitution (USP Hypromellose 2906). Preliminary studies show the method can be applied to the determination of other types of HPMC (Turowski et al., in press).

2. Materials and methods

2.1. Apparatus

Liquid chromatographic system consisting of a Perkin–Elmer Model 200 pump with an autosampler, an ERC-7515A differential refractive index (RI) detector, and a ThermaSphere TS-430 column temperature control device was employed during the study. Waters Corporation BioSuite™ 250, 4 μm UHR SEC, 4.6 × 300 mm, LC column, Waters Corporation Protein-Pak™ 125, 3.9 × 20 mm guard column and in-line LC filters (Alltech Associates) were used to achieve the separation. ATLAS chromatography data system, version 2002-R3 from Thermo Electron Corporation, Waltham, MA, USA, was used for computation of chromatographic data. Two shaking water baths to maintain temperatures at 95 ± 2 °C, and 60 ± 1 °C were utilized during the enzymatic digestion.

2.2. Reagents

Deionized water was used in all aqueous solution preparations. All pre-tested enzymes described in AOAC Method 991.43 were obtained from Sigma–Aldrich and were used within 6 months. All buffers and solutions were prepared as described in AOAC 991.43. MES/TRIS buffer described in AOAC 991.43 Section C(i) was adjusted to pH 4.5 with 6 molar hydrochloric acid and was used as a mobile phase for chromatographic separation. All other reagents were analytical grade purchased either from VWR Scientific or Sigma–Aldrich. Methyl Cellulose (CAS# 9004-67-5) and hydroxypropyl methyl cellulose (CAS# 9004-65-3) were obtained from The Dow Chemical Company as METHOCEL™[1] Cellulose ether A4C and METHOCEL™ Cellulose ether F4M, respectively.

2.3. Preparation of foods

MFG was incorporated in matrices representing meat, vegetable, fruit, bread, and dairy food products, prior to any baking, grinding or drying needed for preparation of the food samples. A bread sample containing no MFG was also prepared for use as a matrix blank. Weights were carefully recorded at each step of sample preparation so that the MFG concentrations could be determined in the dry powders as well as in the original food products.

2.4. Preparation of calibration standard

The MC and HPMC were dried at 105 ± 5 °C for 2 h and 10 mg MFG /g stock solution in pH 8.2 MES/TRIS buffer was prepared by dispersing the accurately weighed amount in the buffer at ~80 °C. This dispersed solution was cooled in the refrigerator at 4 °C for a minimum of 12 h to get a clear solution. This solution was never exposed to temperatures above 40 °C and discarded after 2 weeks. A 40 g portion of this solution was subjected to the pH and temperature treatments described in AOAC 991.43, however enzymes were excluded for the calibration standard solutions. The final weight of this solution was noted to accurately determine the concentration of MFG in the calibration standard. This treated portion was further refrigerated for 16 h at 4 °C.

2.5. Preparation of test solution

A test portion of food containing equivalent to 40 mg MFG was weighed and dispersed in 40 g of pH 8.2 MES/TRIS buffer. The test sample solution was subjected to all the enzymatic, pH and temperature treatments described in AOAC 991.43. The weight of solution at the end of each treatment was noted. Before liquid chromatographic analysis, all solutions were refrigerated for a minimum of 16 h and analyzed within 72 h.

2.6. Preparation of food blank solution

The food blank without MFG and the food containing MFG were prepared with identical procedures. The blank sample was used to correct the background contribution of SDF to the food. Test blank solution was prepared by weighing a food blank portion equal in size to the corresponding food sample and subjecting it to all the enzy-

[1] METHOCEL™ is a trademark of The Dow Chemical Company.

matic, pH and temperature treatments described in AOAC method 991.43. The weight of solution at the end of each treatment was noted. Before liquid chromatographic analysis of the solution it was refrigerated for a minimum of 16 h and analyzed within 72 h. Blank samples, when required, were analyzed concurrent with the corresponding food sample.

2.7. Liquid chromatographic procedure

A 70-μL volume of solution was injected on the constant temperature column set at 25 ± 1 °C, and RI detector set at 35 ± 1 °C. MES/TRIS buffer described in AOAC 991.43 Section C(i) adjusted to pH 4.5 was used as a mobile phase for chromatographic separation. An isocratic flow rate of 0.4 mL/min was used for 25 min.

2.8. Calibration of the liquid chromatograph

A 2-mL portion of the calibration standard solution was filtered into a LC vial using a 0.45-μm-PTFE syringe filter. The solution was injected onto the liquid chromatograph under the conditions described in the procedure. The peak area of MFG was divided by the concentration of MFG to determine the response factor. The final weight of the calibration solution was used to calculate the concentration of the calibration standard.

2.9. Determination of MFG and blank test solution response

Each test and blank solution was filtered using a 0.45-μm-PTFE syringe filter into a LC vial and was injected onto the liquid chromatograph under the conditions described in the procedure. Test solutions left at room temperature for more than 48 h were discarded. The peak area of the blank test solution was subtracted from the peak area of MFG in the corresponding test solution. This corrected response was divided by the response factor to give the concentration of MFG in the test solution. The content of the MFG in the test food sample was determined by taking into account the concentration of MFG in the test solution, the weight of the final test solution, and the weight of the test food sample.

3. Results and discussion

Results obtained during method optimization and during the validation show that post-digestion samples are stable in a refrigerator for several days. The method requires refrigerating digested samples for 16 h to fully hydrate the MFG. Analyses of split samples indicate samples can be refrigerated 2–3 days after digestion with little effect on the recovery. Samples were stable at room temperature for 2–3 days after refrigeration, which facilitates LC analyses using an autosampler. However, samples kept at room temperature more than 3 days after refrigeration show reduced response and should be discarded. For best results, samples should be analyzed as soon as practical after the digestion and refrigeration steps.

The effect of sample size was investigated by analyzing increasingly larger food samples. RI detector response was linear ($R^2 = 0.9998$) versus sample size for up to 10 g samples of bread containing 0.5% HPMC. However, using too large a sample of dried, powdered food will form a paste and make sample preparation difficult. Large samples could also potentially overwhelm the ability of the enzymes to digest the starches and proteins in the food, which might interfere with the analyte response. Consequently, a 5 g maximum sample size was imposed in the method even though larger sample sizes were shown to produce valid results.

Four BioSuite LC columns from two different manufacturing production lots were tested during method optimization and this validation. Numerous guard columns were also employed. Variability resulting from the columns and from the column lots could not be distinguished from the sample-to-sample or day-to-day variability of the method, as determined by monitoring response of the calibration sample.

A series of solutions containing 0.5%, 0.25%, 0.125%, 0.062% and 0.031% of MC were prepared through successive dilution of a stock solution. The LC-RI detector response for MC was determined by analyzing them under the experimental conditions described in the procedure. Solutions were injected in triplicate and the integrated peak area was plotted versus MC concentration. Excellent linearity was achieved down to the 0.031% range ($R^2 = 0.9999$). The same experimental process was followed for HPMC and yielded excellent linear correlation ($R^2 = 0.9998$) down to 0.031%.

Instrument precision was determined through 10 consecutive injections of a 10 mg/g HPMC standard with all instrumental parameters held constant. A relative standard deviation of 0.92% was observed at this level.

Method precision was determined through 12 replicate analyses for each sample. Estimates of within-day and between-day deviation were obtained by performing four of 12 replicates on one day and then performing four replicates on each of two alternate days. In many cases a single

Table 1
Method standard deviation within-groups (Sw), between-groups (Sb), and total (St)

Analyte-matrix sample	Sw	Sb	St	Wt% MFG[a]
Potato starch – MC	0.003	0.01	0.01	0.20%
Milk powder – MC	0.07	0.09	0.1	1.9%
Bread – MC	0.1	0.1	0.2	8.0%
OJ – MC	0.1	0.2	0.2	8.2%
Fish – MC	0.6	2	2	27%
Milk powder – HPMC	0.04	0.03	0.05	0.50
OJ powder – HPMC	0.07	0.1	0.1	0.8
Bread – HPMC	0.04	0.05	0.06	0.84%
Potato starch – HPMC	0.06	0.1	0.1	1.9%
Fish – HPMC	0.2	0.1	0.2	3.8%

[a] MFG concentration in the dried, homogenized food sample.

analyst performed all 12 replicate analyses for a given food sample, but for several samples a different analyst performed the replicates on each of the three alternate days. Table 1 summarizes method precision as within-day (Sw), between-day (Sb) and total (St) deviation for each food matrix tested. The concentration of MFG in each sample is also shown in Table 1. Total standard deviation for the method ranged from 0.01 for potato starch containing 0.2% MC up to 2 for fish containing 27% MC. The relative standard deviations ranged from 0.1% to 7%.

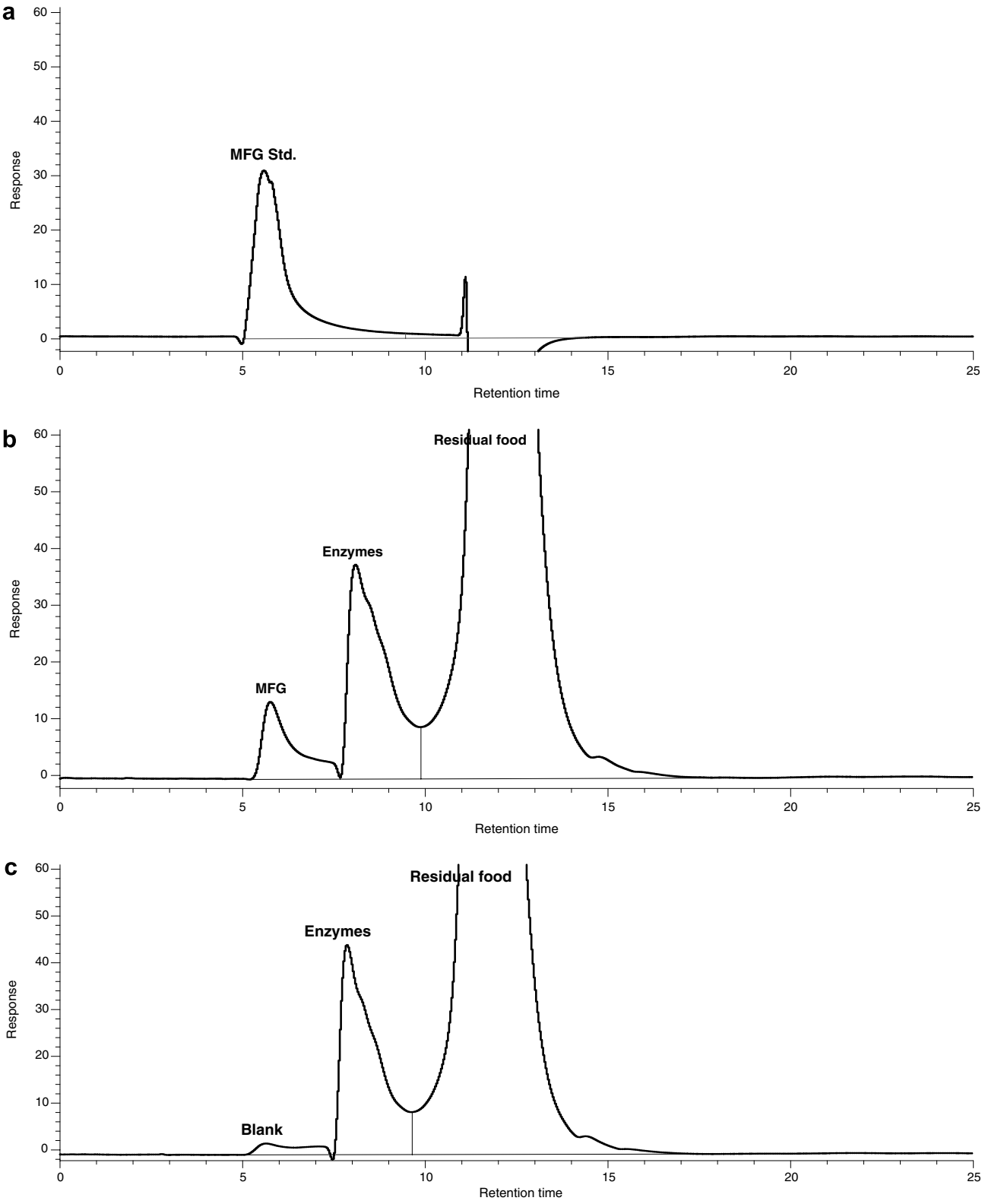

Fig. 1. Chromatograms from (a) calibration solution, (b) bread containing 0.5% HPMC, (c) bread with no HPMC.

Table 2
%Recovery for MC and HPMC in food by the proposed method

Food/analyte matrix	Wt% MFG	Recovered, %[a]	%RSD
Potato starch/MC	0.2%	85	5.2
Milk powder/HPMC	0.5%	98	9.5
Orange juice mix/HPMC	0.8%	113	12
Baked bread/HPMC	0.8%	85	8.0
Milk powder/MC	1.9%	78	6.9
Potato starch/HPMC	2.0%	95	6.0
Fish/HPMC	3.8%	75	7.9
Baked bread/MC	8.0%	84	2.7
Orange juice mix/MC	8.2%	83	2.6
Fish/MC	27%	76	7.1

[a] Average of 12 replicates analyzed over 3 days.

Accuracy of the method was validated by determining percent recovery of MFG from known concentrations of analyte in each food matrices. Quantitation is performed by comparison of the MFG response to a calibration solution analyzed concurrent with the sample. If the food matrix contains fibers which co-elute with MFG under the conditions of this method, quantitation occurs through the additional use of a matrix blank. For example, Fig. 1 shows chromatograms obtained from a calibration solution and from analysis of a bread sample containing 0.5% HPMC. Fig. 1 also presents a chromatogram from a sample of bread blank showing the contribution from resistant starches present in the bread matrix.

Table 2 summarizes the percent recovery for each of the food samples studied. Recovery ranged from 75% for HPMC in fish ($n = 12$), up to 113% for HPMC in Orange juice mix ($n = 12$). Relative standard deviations for each assay ranged from 2.6% to 12%. Results for the two bread samples reported in Table 2 include correction for inherent dietary fiber using a bread blank prepared for the study. Overall recovery of MC was 83% ($n = 60$). Overall recovery of HPMC was 94% ($n = 60$).

The limits of quantitation were determined during method optimization and were found to be 9 μg for MC and 7 μg for HPMC (as 3.3 times the limit of detection).

4. Conclusion

A method is validated for the determination of MC and HPMC in food and food products. There are no current methods suitable for accurate determination of these dietary fibers. Single lab validation studies have shown the method provides sufficient accuracy and precision for the determination of MC and HPMC as SDF in various food and food products. Blank correction can be used, when necessary, to correct for food matrix contributions. A collaborative study is in progress to determine the suitability of the method for use as an official AOAC test method for MC and HPMC. The method is expected to be applicable to other types of modified cellulose ethers, but this should be validated prior to such alternate applications.

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