

STUDY TITLE

Validation of “Aqueous gel permeation/size exclusion chromatography (GPC/SEC) analysis procedure for selected Avail and Nutrisphere-N products” method following AOAC Guidelines

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CERTIFICATION

We, the undersigned, hereby certify that the work described herein was conducted by EN-CAS Analytical Laboratories (EN-CAS).

**Study Director
Approval:**



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12/12/18
Date

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I. ABSTRACT

The objective of this study was to validate the reference Gel Permeation Chromatography/Size Exclusion Chromatography (GPC/SEC) method for use in enforcement of label content claims for Verdesian's polymeric products. These products are based on a maleic-itaconic acid co-polymer with a selected cation: either ammonium, calcium, or sodium, depending on the intended application of the product. The specific utility of this aspect of the enforcement method¹ is limited to qualitative verification of the presence of the polymer, and the quantitative determination of the concentration of propylene glycol, if present, in the polymer product.

The overall method depends on qualitative confirmation of polymer via GPC/SEC, quantitative determination of the product water/solvents content, with the percentage of polymeric salt present being determined by difference.

Although the stated purpose of this chromatographic method is limited to confirmation of polymer presence and quantification of propylene glycol, the validation effort was extended to the monomeric starting materials, maleic and itaconic acid in order to demonstrate their resolution from the polymer and propylene glycol. That said, quantitative determination of the monomers remains beyond the beyond the scope of the method as currently written. The validation further demonstrated the normal chromatographic behavior of the PEG molecular weight standard, the monomeric acids present as unreacted starting materials, and ethylene and propylene glycol. Linearity of response was seen for each of the entities tested and the corresponding Limits of Detection (LOD) were determined.

The validation was conducted at EN-CAS Analytical Laboratories (EN-CAS), Winston-Salem, NC, using the Verdesian method, entitled Aqueous gel permeation/size exclusion chromatography (GPC/SEC) analysis procedure for selected Avail and Nutrisphere-N products (Appendix I). Analyses were performed by liquid chromatography (LC) using refractive index (RI) detection.

The validation demonstrated that the GPC/SEC method is capable of separating the samples into their key components, demonstrating the presence of polymer, and quantification of propylene glycol. The validation effort therefore satisfied the objectives of the study.

¹ Mazo, J., and Orr, G.R. "Enforcement Analytical Methodology for NutriSphere and Avail Polymeric Fertilizer Enhancement Products" May 22, 2015

II. INTRODUCTION

Gel permeation/size exclusion chromatography (GPC/SEC) is a common method of analysis when the objective of an experiment is to separate and/or identify polymers in solution. Within the column, there are a series of small pores along the surface of the packing material. These pores trap and delay the elution of the smaller compounds, allowing the larger polymers to pass through the column quicker. The mobile phase will eventually push the smaller compounds out of the pores and continue to pass through the column.

This study was conducted at EN-CAS Analytical Laboratories in Winston-Salem, NC, in accordance with EN-CAS Protocol # 14-0051, entitled Validation of “Aqueous gel permeation/size exclusion chromatography (GPC/SEC) analysis procedure for selected Avail and Nutrisphere-N products” method following AOAC Guidelines (Ref. 1).

This report contains information on the following: test materials, experimental details, results and discussion, conclusions, data and example calculations.

III. TEST/REFERENCE SUBSTANCES

A. Test Substances

The test substances were obtained from Verdesian Life Sciences LLC and stored under ambient storage conditions. Additional identifying information regarding the test substances is summarized in the following table.

Product	Cation	Manufacturer	Date Received	EN-CAS E#	Lot or Batch #	Physical Appearance
Avail (for Liquid P Fertilizer)	Ammonium	Specialty Fertilizer Products*	12/4/14	EU8123	DCAS022922008-0003	Brown Liquid
Nutrisphere-N (for Granular N Fertilizer)	Calcium	Specialty Fertilizer Products	12/2/14	EU8118	DCNG02132012-0011	Brown Liquid
Nutrisphere-N (for Liquid N Fertilizer)	Calcium	Specialty Fertilizer Products	12/2/14	EU8120	DCNL09032010-0016	Brown Liquid
Avail (for Granular P Fertilizer)	Sodium	Specialty Fertilizer Products	12/2/14	EU8119	DCAG11092012-0027	Brown Liquid

* Specialty Fertilizer Products is a Verdesian Life Sciences company

B. Reference Substances

The reference materials were obtained from a commercial supplier and stored under ambient storage conditions. All reference materials were used to prepare standard solutions for in-lab fortifications and instrumental analysis. Additional identifying information regarding the reference materials is summarized in the following table.

Test Article	Grade	Source	Date Received	EN-CAS E#	% Purity	Lot or Batch #	Exp. Date	Physical Appearance
Propylene Glycol	Analyt.	Sigma-Aldrich	1/5/15	EU8262	99.98	BCBJ5678V	7/15	Clear Liquid
Ethylene Glycol	Analyt.	Sigma-Aldrich	12/30/14	EU8240	99.5	BCBM6058V	7/17	Clear Liquid
Polypropylene Glycol 3000	Analyt.	Sigma-Aldrich	12/30/14	EU8242	~50	BCBL4693V	1/17	Clear Liquid
Maleic Acid	Analyt	Sigma-Aldrich	12/30/14	EU8241	99.0	SLBL3661V	10/22	Crystal
Itaconic Acid	Analyt	Sigma-Aldrich	12/30/14	EU8243	99.0	MKBQ6334V	6/17	Powder

A record of reference substance receipt, storage conditions, and a record of use will be maintained at EN-CAS Analytical Laboratories.

IV. EXPERIMENTAL PROCEDURES

A. Receipt and Storage of Samples

All standard and sample materials that were used for the making of standard solutions, were stored under ambient storage conditions. All standard and test solutions were stored in 12-mL amber vials and under ambient storage conditions.

The samples were received at EN-CAS from Verdesian Life Sciences LLC on 12/2/14 and 12/4/14. Upon receipt, each sample was assigned a unique identification number (E#) and were stored at room temperature in the original shipping packaging until opened for sampling.

B. Summary of Analytical Methods

The validation of this method was based on the AOAC guidelines¹; the data was collected to demonstrate Linearity, Accuracy, Repeatability, Selectivity, Matrix Scope, and the Limit of Quantitation (LOQ)/Limit of Detection (LOD) levels. Linearity was demonstrated by producing a standard calibration curve for each of the analytes: propylene glycol, PEG 3000, itaconic acid, and maleic acid. Each standard calibration curve had to produce a trend line with an R^2 greater than or equal to 0.985. Accuracy was assessed for 1,2-Propanediol (PG) in NutriSphere-N through analyses of neat product and product fortified with three concentrations of PG corresponding to an additional 50, 100 and 150% presumed found in neat product. Multiple injections of standard calibration curves for each analyte were made in order to demonstrate the method repeatability. To demonstrate selectivity, two combined standards were made and run through the instrument. The first mixture contained propylene glycol, ethylene glycol, PEG 3000, and itaconic acid in a 1:1:1:1 ratio. The second mixture contained propylene glycol, ethylene glycol, PEG 3000, and maleic acid in a 1:1:1:1 ratio. Matrix scope was demonstrated by analysis of samples containing either ammonium, calcium, or sodium cations. The diluent/mobile phase was 0.1M sodium nitrate throughout this study. The LOQ and LOD levels were determined by diluting the samples and standards to the point where the specific peak disappears or becomes part of the noise of the mobile phase. The method of analysis included a procedure to prepare the instrumentation before analysis of the standards and samples. To prepare the instrument, the system reference cell was purged for 15 minutes to remove any salts which could have formed while the system was idling, and to auto-zero the Differential Refractometer before the run began. A 0.05% w/w ethylene glycol solution was used to determine system suitability. Afterwards, standards and samples were injected in duplicate. For purposes of quantitation, peak areas must agree within 3%. Suitability was again demonstrated at the completion of analysis. The samples were prepared by weighing out approximately 3 g of the mobile phase, then adding 2-3 drops of the sample, which should weigh roughly 0.032 g (note: the exact weight of sample is to be recorded and used in the calculation), and finally continue to add mobile phase until the absolute concentration of the solution is 0.5%.

¹ AOAC Guidelines for Single Laboratory validation of chemical Methods for Dietary Supplements and Botanicals.

C. Chromatographic System

The instruments that were used in this study are as stated below:

- Waters 717 Autosampler
- Waters 600E System controller
- Waters 600E Pump
- Waters 410 Differential Refractometer
- Waters Ultrahydrogel 120 7.8 x 300 mm column

Waters Corp. Headquarters is located in Milford, MA 01757.

D. Statistical Methods

Statistical analyses used in the study determined the mean and standard deviation of the data collected. All calculations were made using Microsoft Excel.

V. CALCULATIONS

Average:

$$\bar{x} = \sum (\bar{x}) / n$$

Standard Deviation (STDEV):

$$S = \sqrt{\left[\frac{\sum (x_i - \bar{x})^2}{n - 1} \right]}$$

Linear Regression:

$$y = mx + b$$

$$x = \frac{y - b}{m}$$

End Use Product (EUP) Concentration of sample:

$$EUP \text{ concentration} = Soln. \text{ concentration} \times (1/EUP \text{ ratio})$$

$$EUP \text{ ratio} = \frac{Mass \text{ of product}}{mass \text{ of solution}}$$

Concentration of co-polymer in soln.:

$$Conc. \text{ of co - polymer in soln.} = Conc. \text{ of soln.} \times \frac{\% \text{ polymer in original product}}{100\%}$$

Example Calculations:

Concentration of propylene glycol:

$$x = \frac{102467 - 25787}{5527093}$$

$$x = 0.013873$$

EUP Ratio:

$$1 / 0.00526 = 190$$

EUP Concentration:

$$(0.013873) \times (190) = 2.64\%$$

VI. RESULTS AND DISCUSSIONS

A. Linearity

Calibration curves were constructed for each analyte standard, so the concentration of a given analyte may be determined if present in the sample solution. The coefficient of determination (R²) of all calibration curves exceeded 0.985. The following tables and graphs contain the data that was used to construct the calibration curve and its equation.

Propylene glycol

Table 1: Linearity of propylene glycol.

Concentration (% w/w)	Rep	Retention Time (min.)	Area ($\mu\text{V} \times \text{S}$)	Mean \pm SD
0.01	1	10.268	63656	63191 \pm 1153
	2	10.265	62242	
	3	10.264	62840	
	4	10.265	62242	
	5	10.265	64974	
0.05	1	10.255	305585	312914 \pm 4632
	2	10.256	312423	
	3	10.256	314826	
	4	10.251	313548	
	5	10.252	318188	
0.075	1	10.257	446073	468097 \pm 12370
	2	10.26	471660	
	3	10.258	474954	
	4	10.257	473920	
	5	10.258	473877	
0.10	1	10.26	561564	560313 \pm 4242
	2	10.26	552800	
	3	10.259	561681	
	4	10.26	562504	
	5	10.26	563014	
0.25	1	10.257	1406858	1405060 \pm 2393
	2	10.256	1405495	
	3	10.255	1403113	
	4	10.259	1407716	
	5	10.256	1402120	

Table 1 (continued): Linearity of propylene glycol.

Concentration (% w/w)	Rep	Retention Time (min.)	Area (μV x S)	Mean ± SD
0.55	1	10.242	2595502	2664889 ± 31219
	2	10.245	2663237	
	3	10.247	2679215	
	4	10.248	2677245	
	5	10.249	2682292	
	6	10.248	2680146	
	7	10.249	2676585	

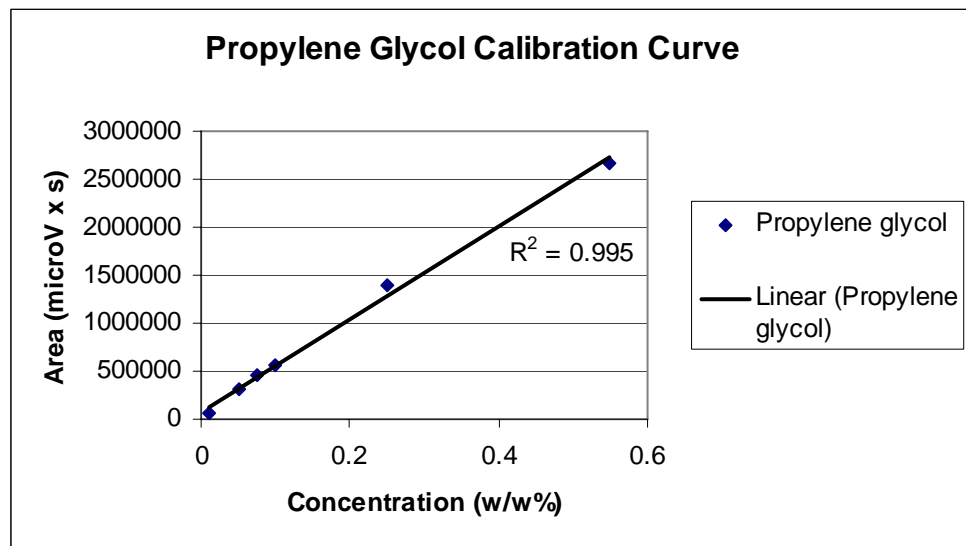


Figure 1: Linearity of propylene glycol. The equation for the trend line is $y = 4787909x + 86496$ using mean points.

PEG 3000

Table 2: Linearity of PEG 3000.

Concentration (% w/w)	Rep	Retention Time (min.)	Area ($\mu\text{V} \times \text{S}$)	Mean \pm SD
0.05	1	6.577	331027	326639 \pm 6703
	2	6.571	315195	
	3	6.573	331064	
	4	6.571	326161	
	5	6.571	329750	
0.075	1	6.573	498377	499901 \pm 6126
	2	6.572	503667	
	3	6.573	492200	
	4	6.574	508081	
	5	6.575	497181	
0.10	1	6.576	597104	602628 \pm 9339
	2	6.576	599616	
	3	6.575	599128	
	4	6.577	598049	
	5	6.574	619243	
0.25	1	6.584	1603232	1601464 \pm 3433
	2	6.583	1604683	
	3	6.584	1596406	
	4	6.581	1599491	
	5	6.582	1603509	
0.50	1	6.588	3309399	3313179 \pm 4504
	2	6.588	3318561	
	3	6.588	3320657	
	4	6.587	3309766	
	5	6.588	3311005	
	6	6.586	3311607	
	7	6.586	3311255	

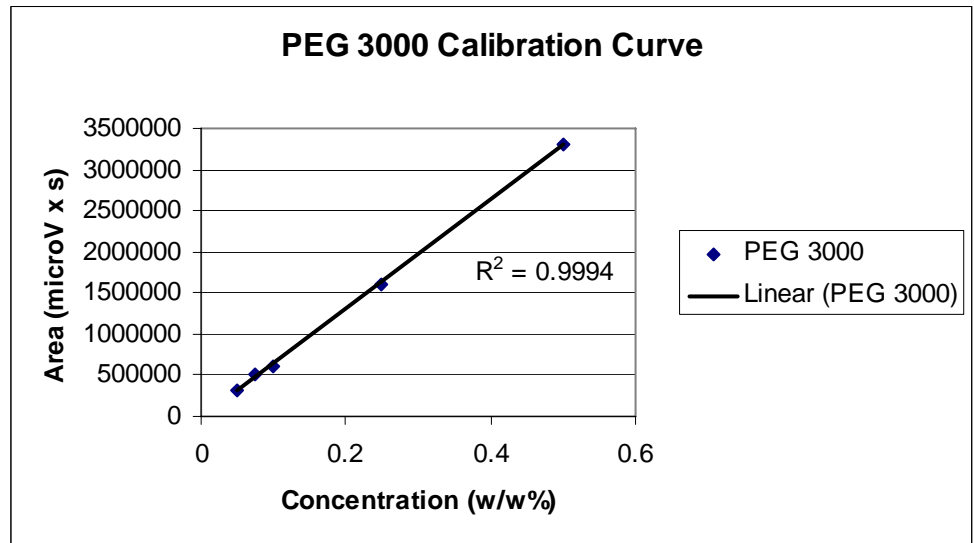


Figure 2: Linearity of PEG 3000. The equation for the trend line is:
 $y = 6647683x - 27536$ using mean points.

Maleic acid

Table 3: Linearity of maleic acid.

Concentration (% w/w)	Rep	Retention Time (min.)	Area ($\mu\text{V} \times \text{S}$)	Mean \pm SD
0.05	1	9.053	476553	498185 \pm 12115
	2	9.061	503428	
	3	9.068	504213	
	4	9.079	504276	
	5	9.086	502455	
0.075	1	9.000	700741	710854 \pm 40489
	2	9.010	644356	
	3	9.021	729583	
	4	9.030	741692	
	5	9.040	737896	
0.10	1	8.953	751057	750963 \pm 3827
	2	8.964	754182	
	3	8.973	748591	
	4	8.982	755083	
	5	8.990	745903	
0.25	1	8.802	1650962	1923812 \pm 184235
	2	8.830	1813282	
	3	8.853	2059824	
	4	8.874	2049804	
	5	8.896	2045190	
0.50	1	8.489	3145887	3080765 \pm 46591
	2	8.360	3122013	
	3	8.575	3110038	
	4	8.614	3072807	
	5	8.653	3058520	
	6	8.691	3035387	
	7	8.727	3020700	

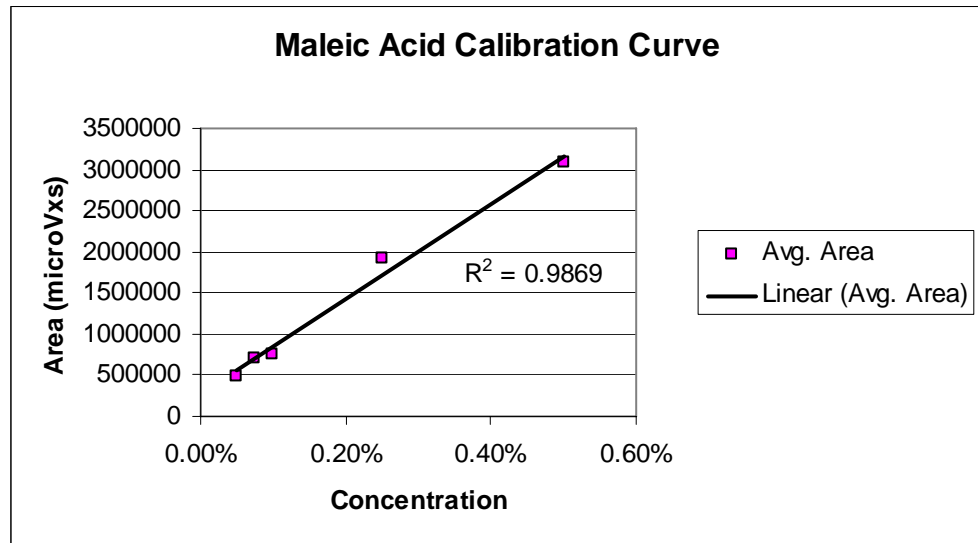


Figure 3: Linearity of maleic acid. The equation for the trend line is: $y = 5811830x + 259609$ using mean points.

Itaconic acid

Table 4: Linearity of itaconic acid.

Concentration (% w/w)	Rep	Retention Time (min.)	Area ($\mu\text{V} \times \text{S}$)	Mean \pm SD
0.05	1	8.017	479034	487357 \pm 15716
	2	8.013	515428	
	3	8.011	480487	
	4	8.009	480366	
	5	8.007	481468	
0.075	1	8.018	759464	732956 \pm 27251
	2	8.017	752354	
	3	8.018	689550	
	4	8.019	733157	
	5	8.020	730254	
0.10	1	8.006	932478	943739 \pm 6484
	2	8.010	946595	
	3	8.011	946894	
	4	8.014	944190	
	5	8.017	948538	
0.25	1	7.923	2257254	2302110 \pm 64061
	2	7.930	2348569	
	3	7.946	2255350	
	4	7.967	2257463	
	5	7.990	2391916	
0.50	1	7.609	3994042	4025840 \pm 39220
	2	7.646	4010898	
	3	7.694	4002862	
	4	7.748	4009432	
	5	7.802	3998440	
	6	7.851	4081386	
	7	7.901	4083818	

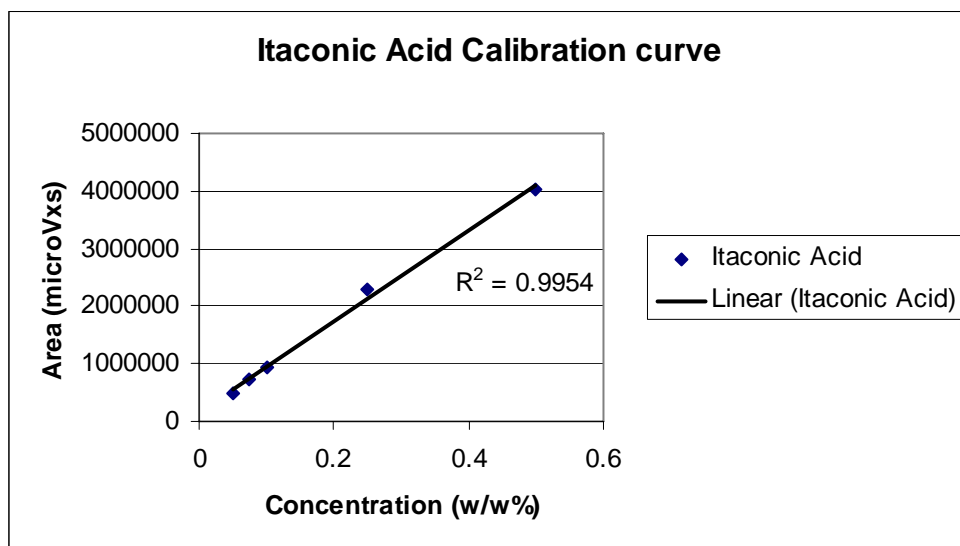


Figure 4: Linearity of itaconic acid. The equation for the trend line is: $y = 7873456x + 163076$ using mean points.

B. Reproducibility

This test for validation required that three nominal concentrations of each analyte standard (0.5, 0.1, and 0.05 %) would be injected into the column 5 times. The reproducibility of the system was determined by measuring the standard deviations from the data collected. The standard deviations were calculated from the retention times. Retention time (RT) is based on the size of the analytes and is used to identify what compound moved through the column at a specific time. The standard deviations for the polymer samples did not exceed ± 0.03 . The standard deviations calculated from the acid data were not greater than ± 0.08 . The following tables show the mean and standard deviations for each analyte standard and the data that was collected.

Table 5: Analyte Mean and Standard deviations.

Analyte std.	MEAN	STDEV
Propylene glycol	10.2419	0.003035
PEG 3000	6.57520	0.008874
Maleic acid	9.32873	0.087203
Itaconic acid	7.73053	0.14903

Table 6: Reproducibility of Propylene glycol.

Propylene glycol		
Concentration (% w/w)	Retention Time (min.)	Area ($\mu\text{V} \times \text{s}$)
0.05	10.245	274176
	10.244	279721
	10.244	278098
	10.239	271855
	10.240	271406
0.1	10.246	544156
	10.244	546658
	10.244	548930
	10.239	555864
	10.240	552669
0.55	10.244	2778702
	10.245	2776914
	10.240	2776607
	10.236	2792265
	10.239	2785410

Table 7: Reproducibility PEG 3000.

PEG 3000		
Concentration (% w/w)	Retention Time (min.)	Area ($\mu\text{V} \times \text{s}$)
0.05	6.581	322943
	6.584	324365
	6.555	335049
	6.566	336499
	6.565	334316
0.1	6.582	602362
	6.584	587212
	6.571	607236
	6.571	607061
0.5	6.571	607813
	6.584	3266380
	6.587	3282255
	6.575	3465902
	6.576	3465237
	6.576	3457874

Table 8: Reproducibility of maleic acid.

Maleic acid		
Concentration (% w/w)	Retention Time (min.)	Area ($\mu\text{V} \times \text{s}$)
0.05	9.430	646984
	9.433	678499
	9.433	589146
	9.297	617410
	9.299	617585
0.1	9.404	878822
	9.410	960296
	9.416	873718
	9.277	981946
0.5	9.284	974577
	9.266	4459976
	9.296	4381464
	9.320	4919431
	9.168	5065317
	9.198	5002775

Table 9: Reproducibility of itaconic acid.

Itaconic acid		
Concentration (% w/w)	Retention Time (min.)	Area ($\mu\text{V} \times \text{s}$)
0.05	7.907	537267
	7.906	536978
	7.624	542913
	7.622	546223
	7.620	546126
0.1	7.915	951244
	7.912	954798
	7.636	931562
	7.626	934632
	7.625	936645
0.5	7.876	4118944
	7.916	4085067
	7.565	4123545
	7.585	4092887
	7.623	4097166

C. Accuracy

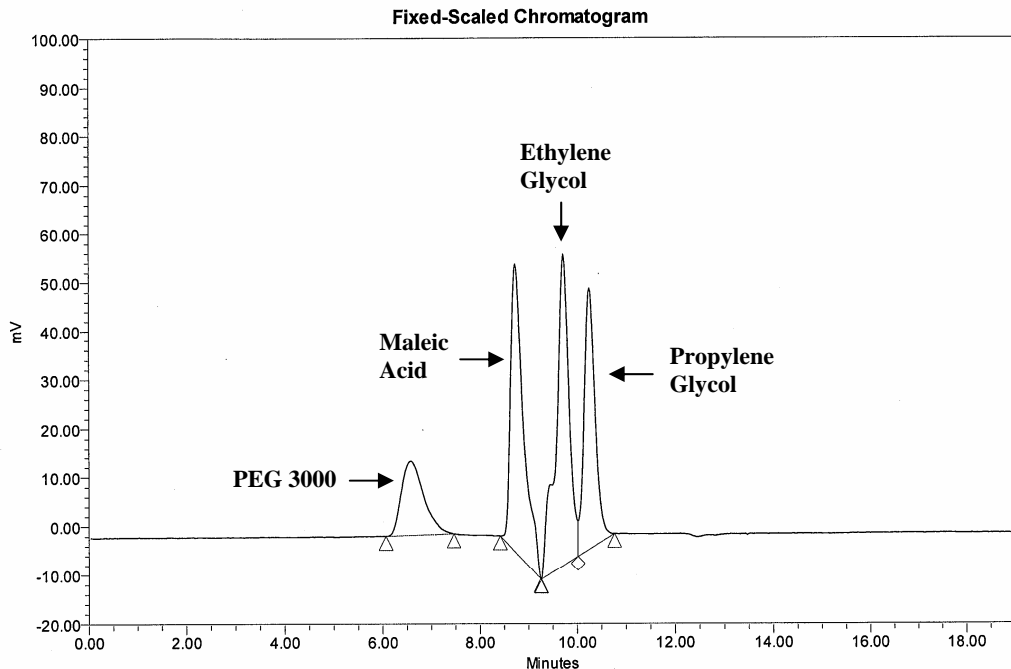
The concentration of 1,2-Propanediol (PG) in NutriSphere-N was analyzed in neat product and product fortified with three concentrations of PG corresponding to an additional 50, 100 and 150% presumed found in neat product. All concentration levels were determined in triplicate. The average percent recoveries for each fortification level were determined after subtracting the amount found in neat product. The net average percent recovered in samples fortified with an additional 50, 100 and 150% were, respectively, 95.7, 93.8 and 90.6 (Table 10). These results demonstrate that the method can be used to accurately determine PG over a range of concentrations.

Table 10: Accuracy of propylene glycol quantitation.

Fortification Level	% Recovery	Mean	% CV
+50%	95.1	95.7	1.10
	97.0		
	95.1		
+100%	93.3	93.8	0.450
	94.2		
	93.8		
+150%	90.6	90.6	0.984
	91.5		
	89.7		

D. Selectivity

The analytical column was able to separate sample solutions into distinct components, with combined solutions (mixed standards) of analyte standards yielding sharp, easily identified peaks. **Figures 5 and 6** show the results of injecting the second and first combined solution, respectively.

**Figure 5:** Combined PEG 3000, maleic acid, ethylene glycol, and propylene glycol standard.

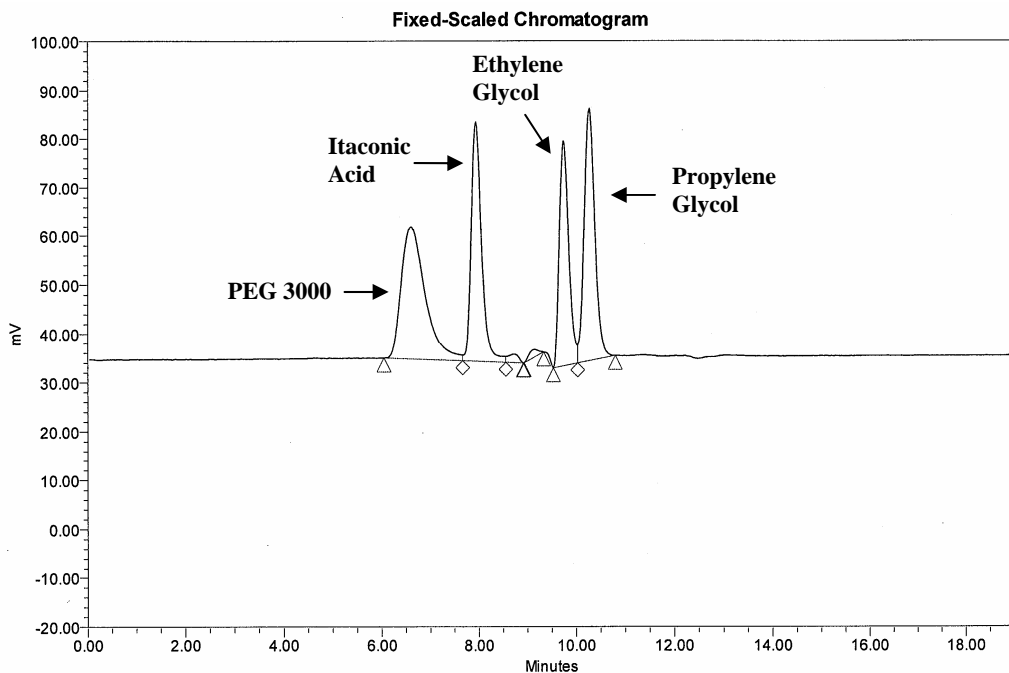


Figure 6: Combined PEG 3000, itaconic acid, ethylene glycol, and propylene glycol standard.

Sample solutions were more complex than the combined standards and produced correspondingly more complex chromatograms. Small, unidentified peaks were observed eluting between itaconic acid and propylene glycol; these peaks did not affect measurement of the peaks of interest. The column was able to separate the maleic-itaconic copolymer from some trailing monomeric acid compounds (Fig. 7), and in sample EU8118 achieved baseline resolution of propylene glycol as well. **Figures 7, 8, 9, and 10** show the results of injecting EU8118, EU8120, EU8123 and EU8119, respectively, into the system.

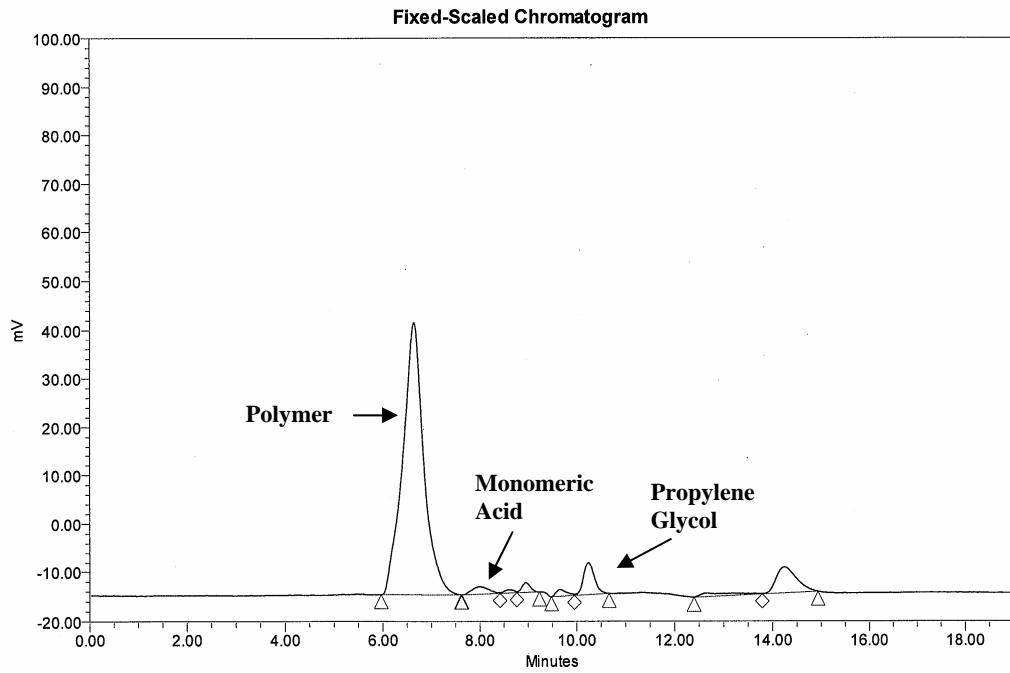


Figure 7: Nutrisphere-N (for granular fertilizer).

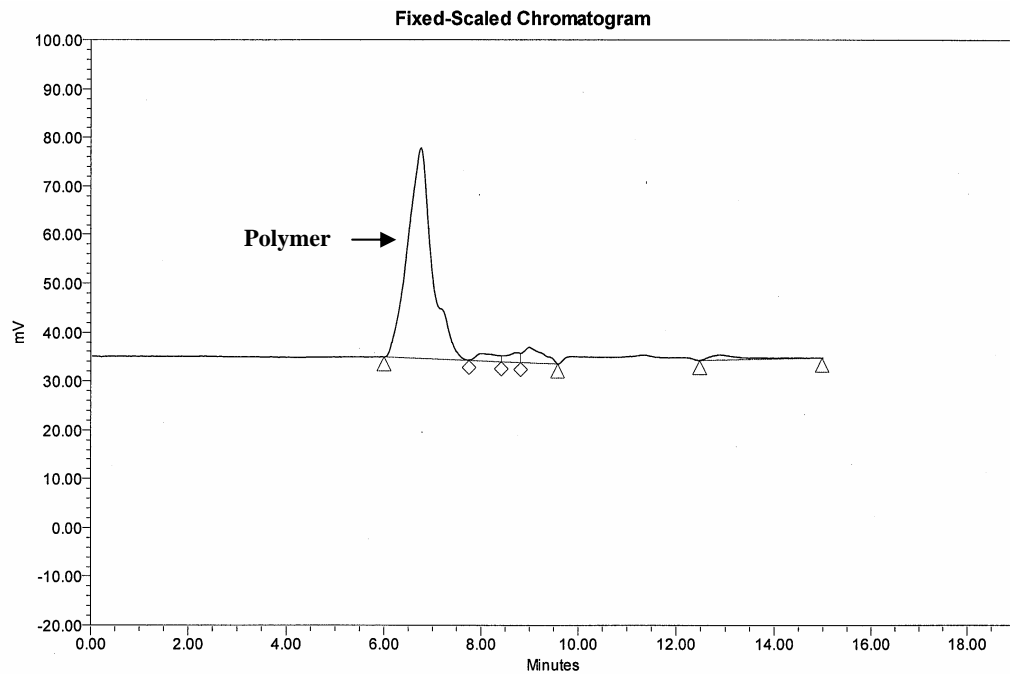


Figure 8: Nutrisphere-N (for liquid fertilizer).

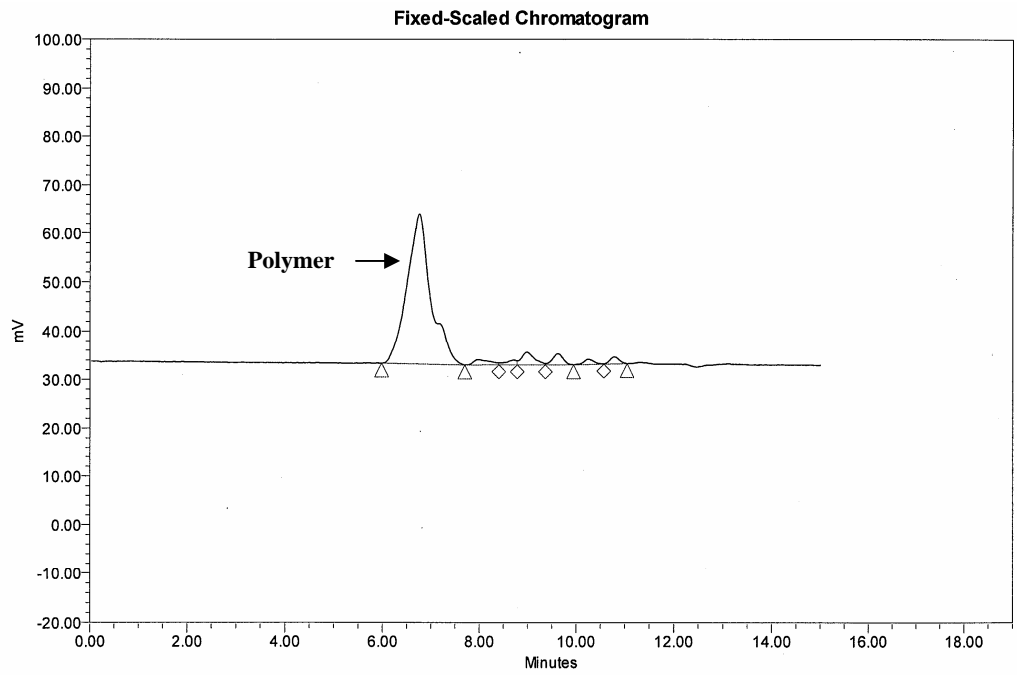


Figure 9: Avail (for liquid fertilizer).

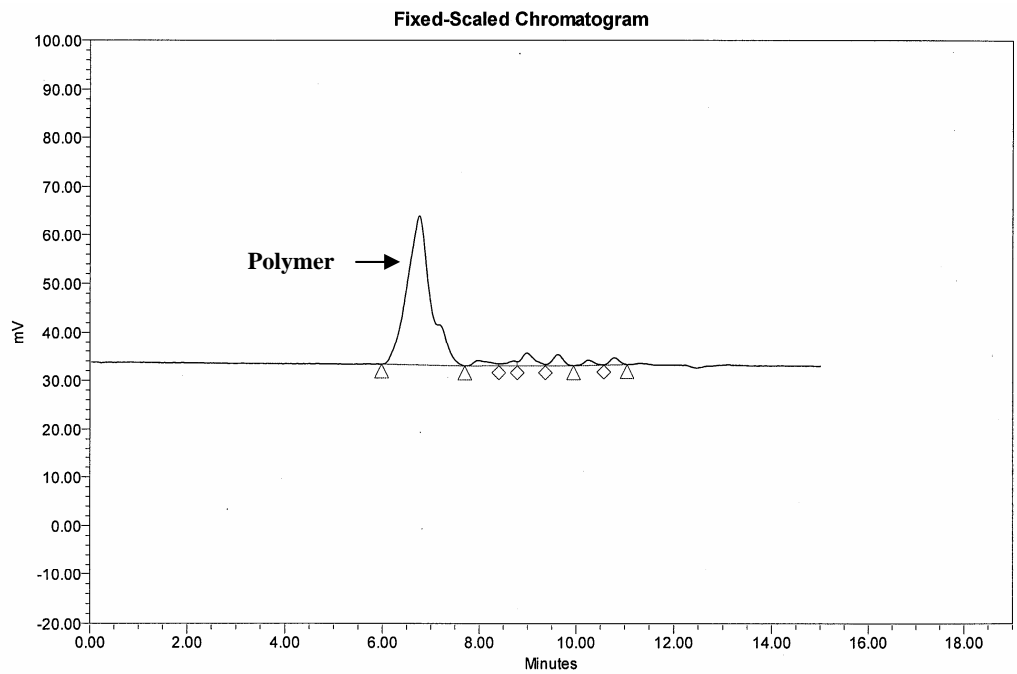


Figure 10: Avail (for granular fertilizer).

E. LOD/LOQ

The Limit of Detection (LOD) was determined by diluting solutions of the products to the lowest concentration that yielded discernible measurements. LODs were 0.00117% w/w, 0.000192% w/w, and 0.00015% w/w for the maleic-itaconic acid co-polymer, propylene glycol, and the monomeric acids. In terms of EUP concentrations, the LODs were 0.2223% w/w, 0.0365% w/w, and 0.0285% w/w. In the absence of certified analytical standards and placebo materials, the method LOQ could not be determined. Therefore, the method should be considered qualitative in nature.

F. Suitability Test

$$N = 5.54 \times (V_R / w_{1/2})^2$$

The reference method states that the analytical column must have a least 13,000 theoretical plates in order to separate sample components. To determine the number of theoretical plates (N) a column has, injections of 0.05% ethylene glycol and the above equation must be used. V_R is equal to flow rate x retention time of the solution, and $w_{1/2}$ is equal to the width of the peak at half its height.

The largest number of theoretical plates that were calculated for the column used in this study was roughly 10,500. Despite the lower number of theoretical plates, the column was still able to successfully separate the co-polymer, monomeric acid, and propylene glycol. (Refer to Figures 5 and 6.)

VII. CONCLUSIONS

This validation exercise demonstrated that the GPC/SEC analytical method used in this study successfully separated Avail and Nutrisphere product components with a at lower number of theoretical plates than stated in the reference method's suitability specification. It was shown to have adequate resolution and demonstrated linearity and reproducibility. The method satisfied AOAC guidelines for all tests required for validation. The data and results presented in this report confirm that this method is suitable for the determination of the presence of the co-polymer and the concentration of propylene glycol in selected Avail and Nutrisphere-N products.

VIII. REFERENCES

EN-CAS Analytical Laboratories in Winston-Salem, NC, in accordance with EN-CAS Protocol # 14-0051, entitled Validation of “Aqueous gel permeation/size exclusion chromatography (GPC/SEC) analysis procedure for selected Avail and Nutrisphere-N products” method following AOAC Guidelines, issued June 6, 2015.

APPENDIX I

Verdesian Method Summary

Aqueous gel permeation/size exclusion chromatography (GPC/SEC)
analysis procedure for selected Avail and Nutrisphere-N products

Aqueous gel permeation/size exclusion chromatography analysis of for selected Avail and Nutrisphere-N products

A. Principle

The presence of a maleic-itaconic acid co-polymer can be detected by gel permeation chromatography using a 0.1M sodium nitrate mobile phase and a Differential Refractometer set at 35°C.

B. Apparatus

- (a) High Performance Liquid Chromatograph - System equipped with refractive index detector and column oven thermostated at 35°C, and sample injection valve with 25 μ L sample loop. Operating conditions: flow rate set at 1.0 mL/min.
- (b) HPLC GPC column – Stainless steel, Waters Ultrahydrogel 120 7.8-m x 300-mm column.

C. Reagents

- (a) Solvents – HPLC grade water
- (b) Mobile phase – 0.1M sodium nitrate solution
- (c) Standard prep – Accurately weigh out 0.125 g of each analyte (propylene glycol, ethylene glycol, PEG 3000, maleic acid, and itaconic acid) and transfer each to separate 25mL volumetric flasks. Add roughly 25 mL of mobile phase and mix well.

D. Sample Prep.

For a 0.5% w/w solution, weigh out roughly 0.032 g of sample for every 6.15 g of mobile phase being used. Then transfer both to a vial and or container of appropriate size, and mix well. Dilute the solution if need be.

E. System Suitability

A system's suitability can be tested two ways:

1. With a 0.05% w/w ethylene glycol solution; make three injections but only use the results of the third injection. Using those results, calculate the number of theoretical plates in the column, if the column contains more than 13,000 theoretical plates then the test is suitable then proceed with the study. If the column has less than 13,000 plates, then proceed to the second test.
2. Demonstrate peak resolution using a 1:1:1:1 PEG 3000:itaconic acid:ethylene glycol:propylene glycol combined standard.

F. Determination

Make multiple 25 μ L injections for each analyte standard at different concentrations. Use the values for area from the results and the concentrations to make linear calibration curves for the standard. This curve will be used to determine the concentration of the analyte present in the sample solution.

Inject samples and calibrants until areas of replicates match within 3%.

The concentration of the propylene glycol is calculated from the solution concentration standard curve then corrected for EUP.

Use the PEG 3000 as a marker for the co-polymer retention time. Presence of a peak at this retention time range verifies the presence of the co-polymer.

For determining the End Use Product (EUP) concentration of the sample in the solution, use the equation below:

$$\begin{aligned} \text{Mass of product} / \text{mass of solution} &= \text{EUP ratio} \\ \text{PG} \times (1 / \text{EUP ratio}) &= \text{EUP concentration of PG} \end{aligned}$$