

Method Title

Enforcement Analytical Methodology for  
NutriSphere and Avail Polymeric Fertilizer  
Enhancement Products

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## Introduction

The determination of active polymer salt content in Nurtisphere N and Avail fertilizer enhancement products for the purposes of label claim enforcement is a two-step process. The first step involves qualitative confirmation of polymer presence and identification of the corresponding counterions. The second step involves quantitative determination of the product water/solvents content with the percentage of polymeric salt present being determined by difference.

Polymer presence is established via aqueous gel permeation/size exclusion (SEC) chromatography as described within. The confirmation of polymer presence is qualitative and not quantitative due to both the properties of the analysis and the nature of the analytes involved.

In his review of SEC, Bernd Trathnigg<sup>1</sup> of the Institute of Chemistry at Karl-Franzens-University Graz, Austria list the difficulties inherent in SEC analyses.

Provided that the separation itself is reliable (which cannot always be taken for granted!), the subsequent transformations are subject to errors:

1. Elution time to elution volume. This requires a highly constant and reproducible flow rate, which means that only high quality pumps should be used.
2. Elution volume to molar mass. The molar mass of a fraction can be obtained either from a calibration or from a molar mass sensitive detector (in addition to the concentration detector).
3. Detector response to polymer concentration. This requires a sufficiently wide linear range, a well-defined response of the detector(s) along the entire peak (i.e. for all molar masses within the molecular mass distribution (MMD)), and – in the case of copolymers – a second concentration detector.

The first two difficulties can be overcome by use of reliable equipment and suitable calibration samples but the third difficulty lies with the physico-chemical properties of the polymer in question. Further in his review Professor Trathnigg very correctly states:

In SEC of polymers, most chromatographers assume a constant response factor within the entire MMD, which is, however, justified only in the analysis of homopolymers with sufficiently high molar mass.”

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<sup>1</sup> Trathnigg, Bernd. "Size-exclusion chromatography of polymers." Encyclopedia of analytical chemistry (2000). R.A. Meyers (Ed.) Copyright John Wiley & Sons Ltd and references contained therein.

He cites the following examples of this presumption failing (**emphasis** added):

In a concentration series of Polystyrenes, **a nonlinear relation between sample size and peak area was found**. Lukyanchikov et al. described similar deviations in the analysis of butadiene–styrene copolymers

Further, this is in part due to what the text calls local polydispersity.

Basically, in SEC there will always be local polydispersity in each slice of the polymer peak: in the case of homopolymers because of peak spreading, in the case of copolymers and polymer blends because of overlapping chemical composition distribution (CCD) and MMD.

The NutriSphere and Avail products are all copolymers that have both MMD and CCD. This is a consequence of the method of manufacture, details of which are both confidential business information and beyond the scope of this introduction.

In this case, suffice it to say that the copolymers in question are too complex in terms of their MMD and CCD to make any assumption of constant response factor. As seen above, even with well-behaved homopolymers of high molecular weight this assumption is not a sure thing, and the purpose of the SEC is purely to demonstrate presence of polymer as claimed on the product labels.

The identities of the counterions sodium, ammonium and calcium are determined through *via* established AOAC methodology.

Quantification of the solids contents of NutriSphere-N for Liquid Nitrogen Fertilizers, Avail for Liquid Phosphate Fertilizers, and Avail for Granular Phosphate Fertilizers, are determined by subtraction of the water value as determined by Karl Fischer titration. The same solids content determination for NutriSphere-N for Granular Nitrogen Fertilizers involves subtraction of both the water content as determined by Karl Fischer and the propylene glycol content as determined by SEC chromatography.

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

**Need, purpose and scope of method:**

This method is used for analysis of Avail® and NutriSphere-N® copolymer-containing formulations. It is intended for confirmation of product polymer content and quantitative analysis of propylene glycol (as applicable) in those formulations.

**Method principle:**

The method uses gel permeation/size exclusion chromatography to separate sample into its components on the basis of molecular size in solution.

**Apparatus and settings:**

The analysis is conducted using a high performance liquid chromatography unit. A typical equipment set up with corresponding run parameters is:

- Pump: model L-2130 (Hitachi LaChrom Elite, Dallas, TX,), 1.00 ml/min.
- Autosampler: model L-2200 (Hitachi LaChrom Elite, Dallas, TX), injection volume 25 microliters.
- Refractive index (RI) detector: model L-2490 (Hitachi LaChrom Elite, Dallas, TX).
- Precolumn filter: model A-316, 0.5 micron (Upchurch Scientific, Oak Harbor, WA)
- Column: Ultrahydrogel 120, 300X7.8 mm, part # WAT011520 (Waters, Milford, MA).
- Data System: EZChrom (Hitachi LaChrom Elite, Dallas, TX)
- Run time: about 15 minutes per injection

Functionally equivalent equipment and/or columns can be substituted for those listed above.

**Reagents:**

The analysis is carried out in the following mobile phase: 0.10 M sodium nitrate (analytical grade, Catalog # 31440, Sigma, St. Louis, MO) in HPLC grade water. Mobile phase is filtered using a suitable inert filter and degassed prior to use. Same mobile phase

is used for standard, sample, and system suitability test solution preparation. Mobile phase may be stored in sealed containers for up to five (5) days.

**Standard, sample, and system suitability solution preparation.**

Standards, samples and system suitability test compound are dissolved in mobile phase. All injections are done in duplicate, checked for peak area reproducibility (peak area reproducibility to within 3.0% of average), and averaged if reproducible. All solutions are prepared by weight no longer than 24 hours before injection. Test sample of product must be stored at room temperature in a sealed container; permissible container materials in contact with sample: borosilicate glass, pure PTFE. System suitability is tested immediately before and immediately after performing analysis of samples. Only analysis results where the system was suitable both before and after analysis are to be used.

*Standards:*

Aqueous polyethylene glycol polymer standard is used (Catalog # 81269, Sigma, St. Louis, MO) to prepare a solution of about 0.10% w/w actives in mobile phase. This is the qualitative polymer standard.

Propylene glycol standard is used (Catalog # 12279, Sigma, St. Louis, MO) to prepare a solution of accurately known concentration (to three significant figures or better), about 0.05% w/w in mobile phase. This is the quantitative propylene glycol standard.

*Samples:*

Products to be tested are dissolved to accurately known concentrations (to three significant figures or better) of about 0.5% w/w on “as is” basis in mobile phase. This is the prepared sample solution.

*System suitability solution:*

Ethylene glycol (Catalog # 03750, Sigma, St. Louis, MO) is used to prepare a solution of about 0.05 % w/w in mobile phase.

**Performing the test:**

System is started up and allowed to suitably equilibrate. Suitability solution is injected. Theoretical plate count (via USP method) for system suitability solution is computed. System is suitable if theoretical plate count is not below 13,000 theoretical plates.

If system is suitable, proceed to inject (in duplicate) propylene glycol quantitative standard and polyethylene glycol qualitative standard. Note peak elution time, and compute average peak height for propylene glycol standard and use it to calculate response factor for propylene glycol. Not all samples have propylene glycol. Note peak elution time for polyethylene glycol peak. It is located approximately in the polymer region of chromatogram.

Inject (in duplicate) prepared sample solutions. Product chromatograms will show polymer peak (not necessarily gaussian) in the polymer region. Certain products that contain propylene glycol will show a separate propylene glycol peak. Peak height should be recorded, averaged, and used to compute propylene glycol content of product based on standard's response factor and sample concentration. Cations present in sample (sodium, ammonium, calcium) may generate additional peak(s) at times specific to individual cations.

After all samples are analyzed, repeat system suitability test. If system suitability test requirements are met, data from experiment may be used.

**Results:**

For each sample, report presence or absence of polymer in chromatogram. For samples containing propylene glycol, report presence of propylene glycol and calculated concentration as % w/w of total product.

## Typical Chromatograms

Figure 1. Ethylene Glycol System Suitability Chromatogram

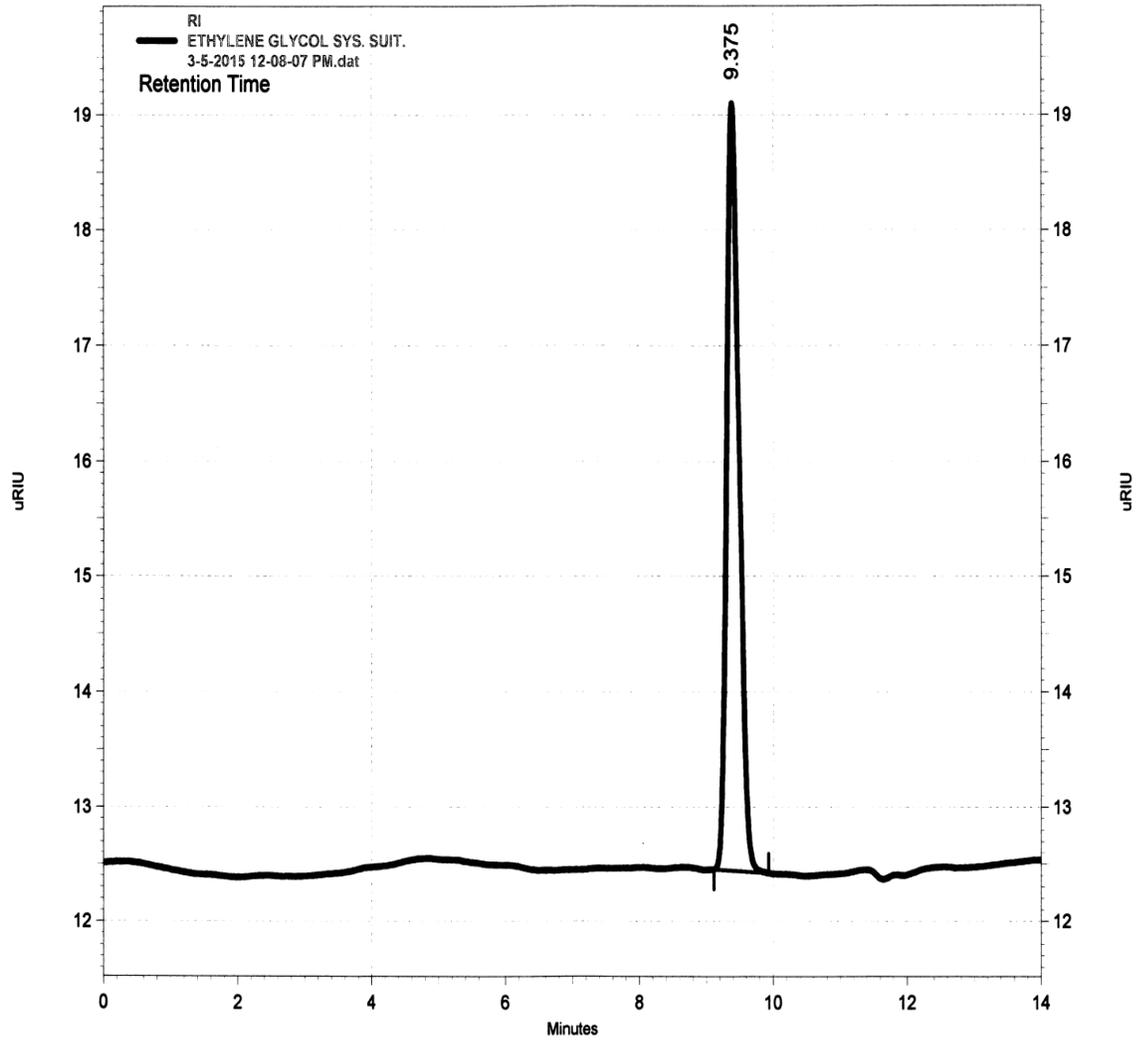


Figure 2. Propylene Glycol Quantification Standard

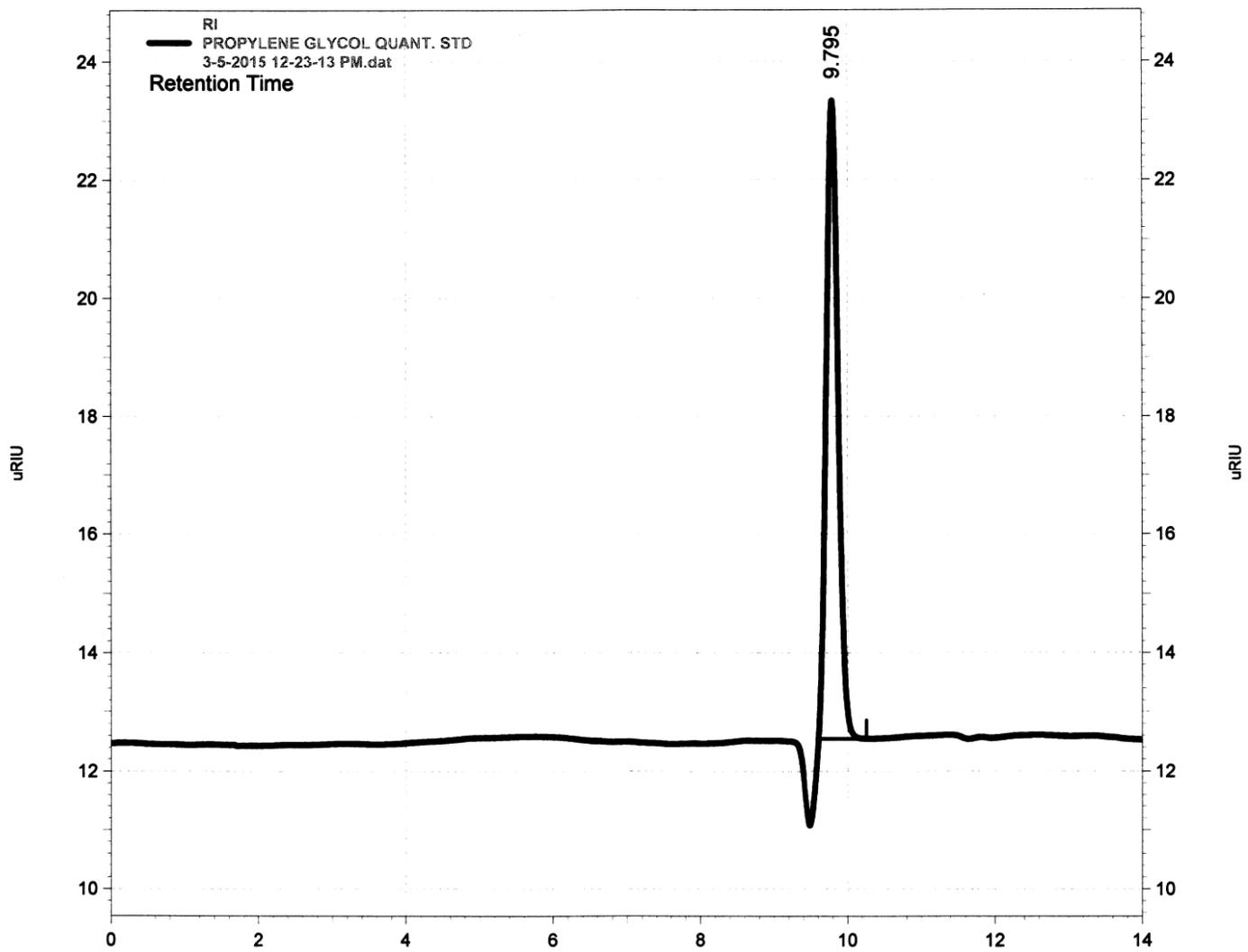


Figure 3. Polyethylene Glycol Quantification Standard

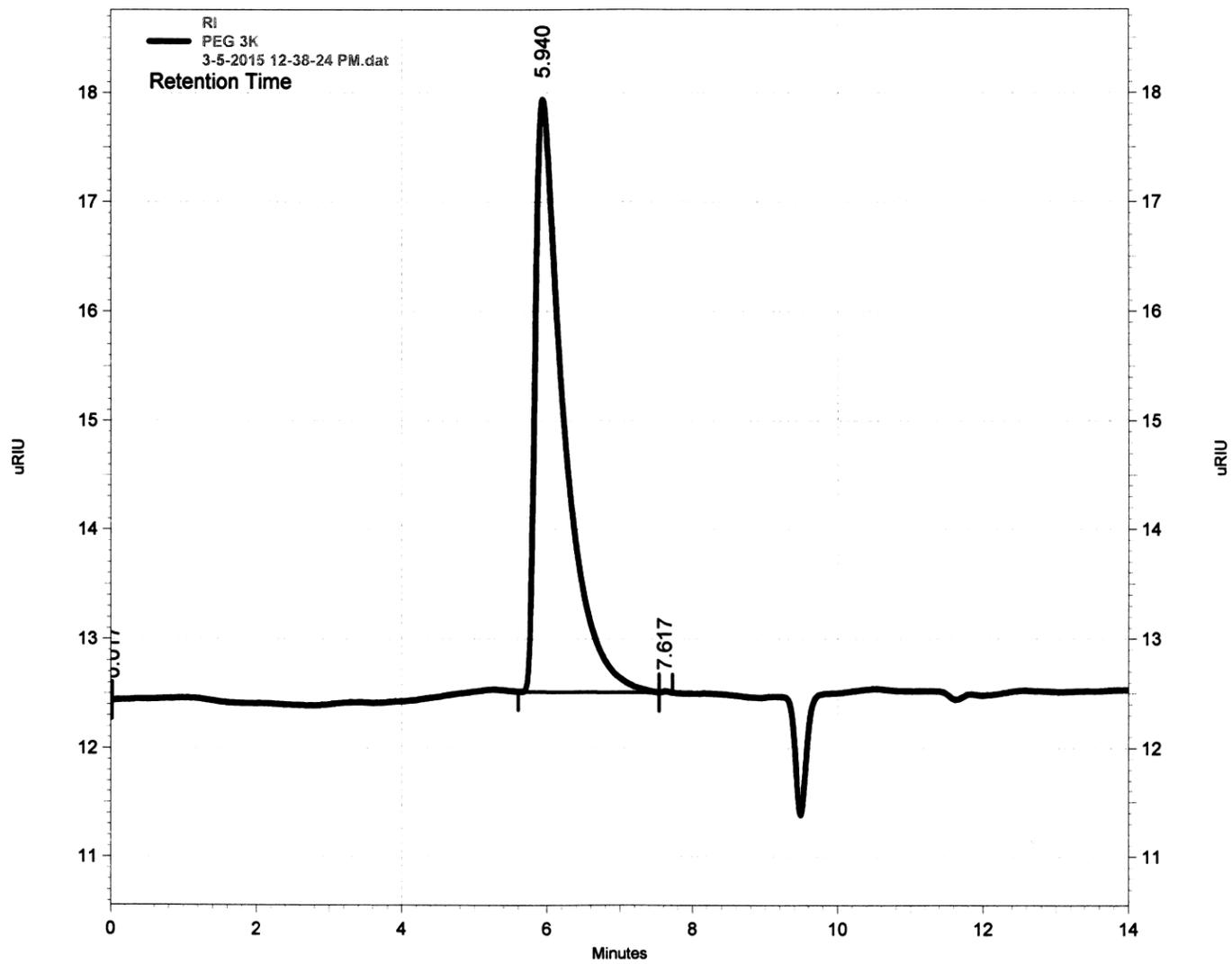


Figure 4. NutriSphere-N for Liquid N Fertilizers

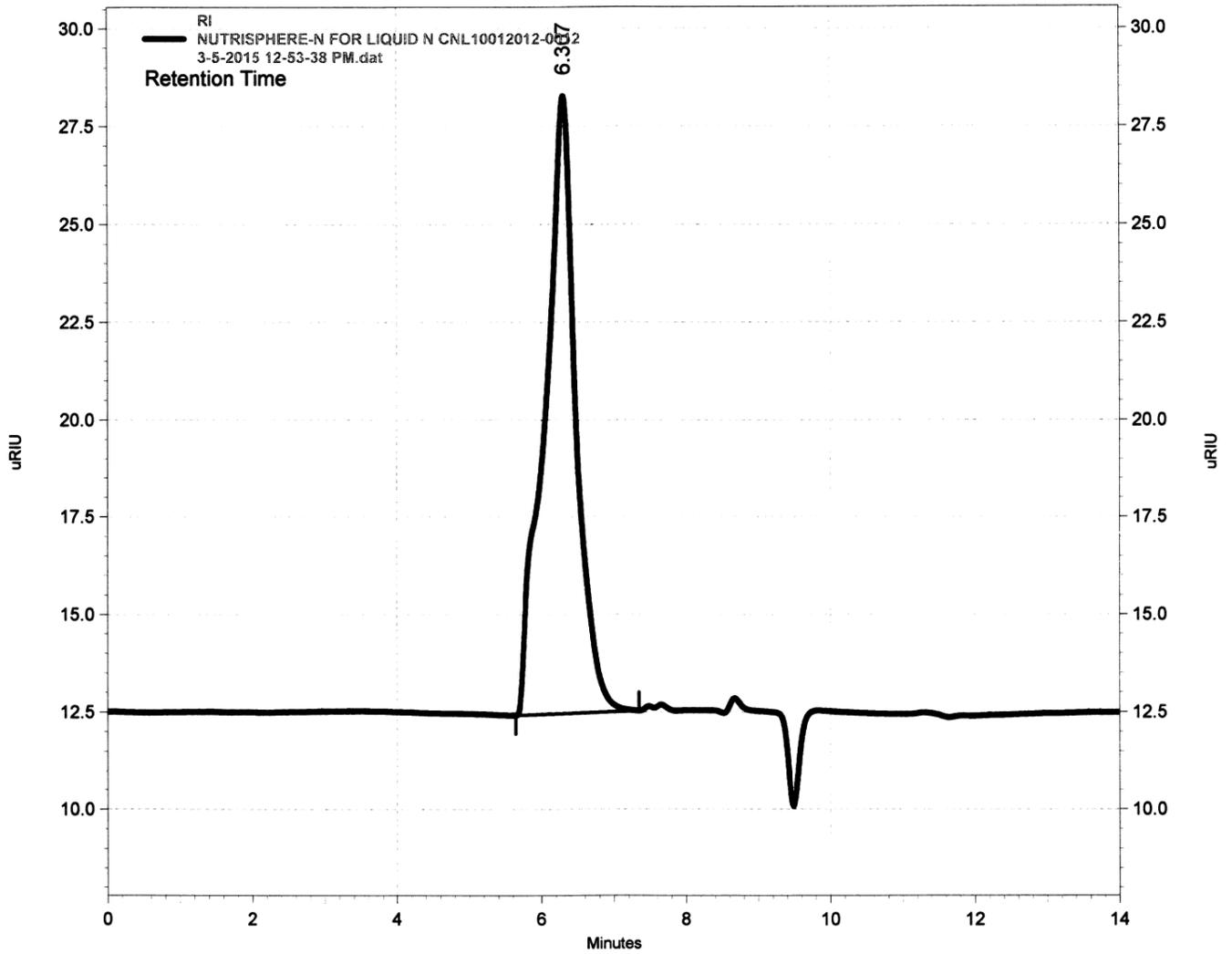


Figure 5. NutriSphere-N for Granular N Fertilizers

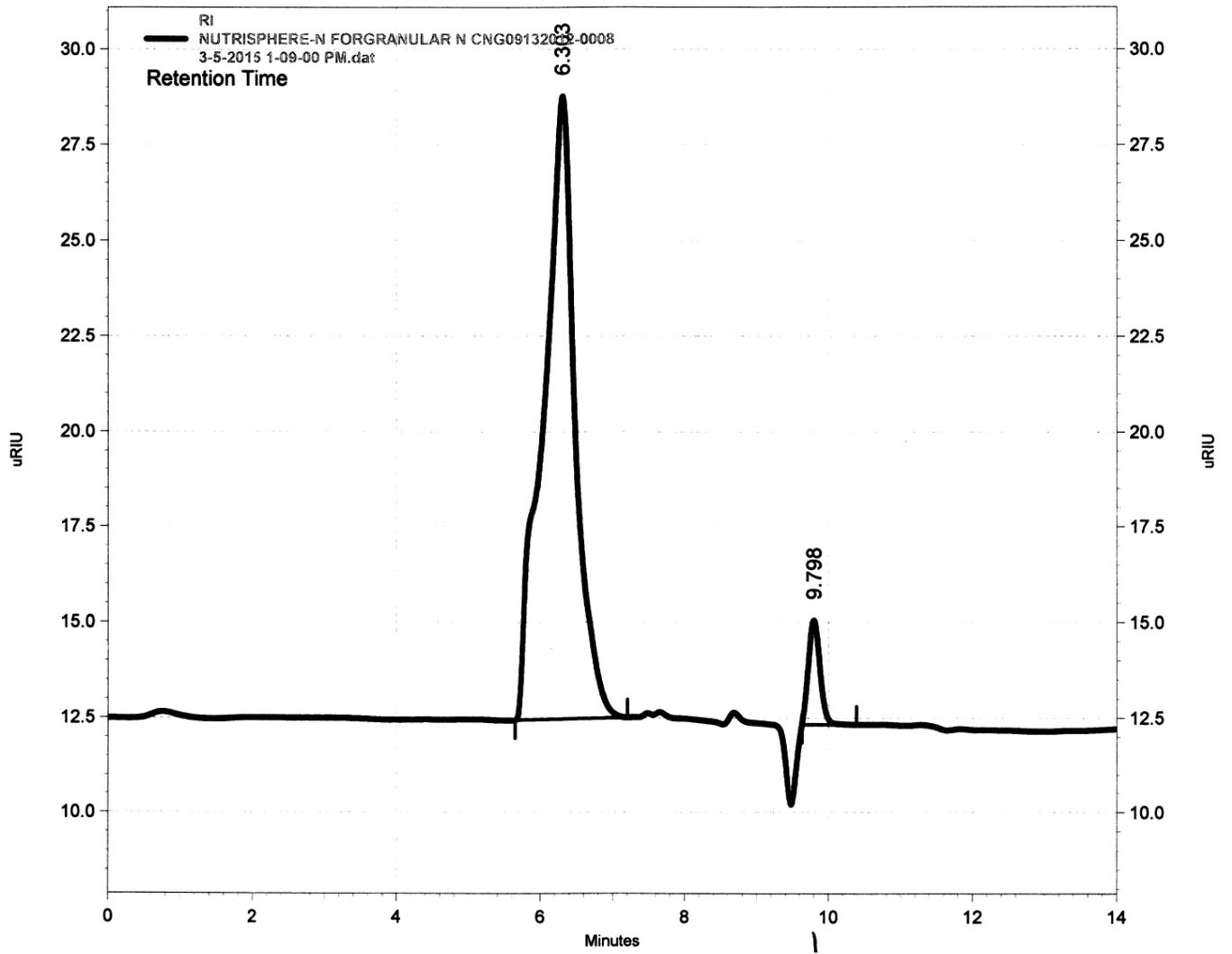


Figure 6. Avail for Granular P Fertilizers

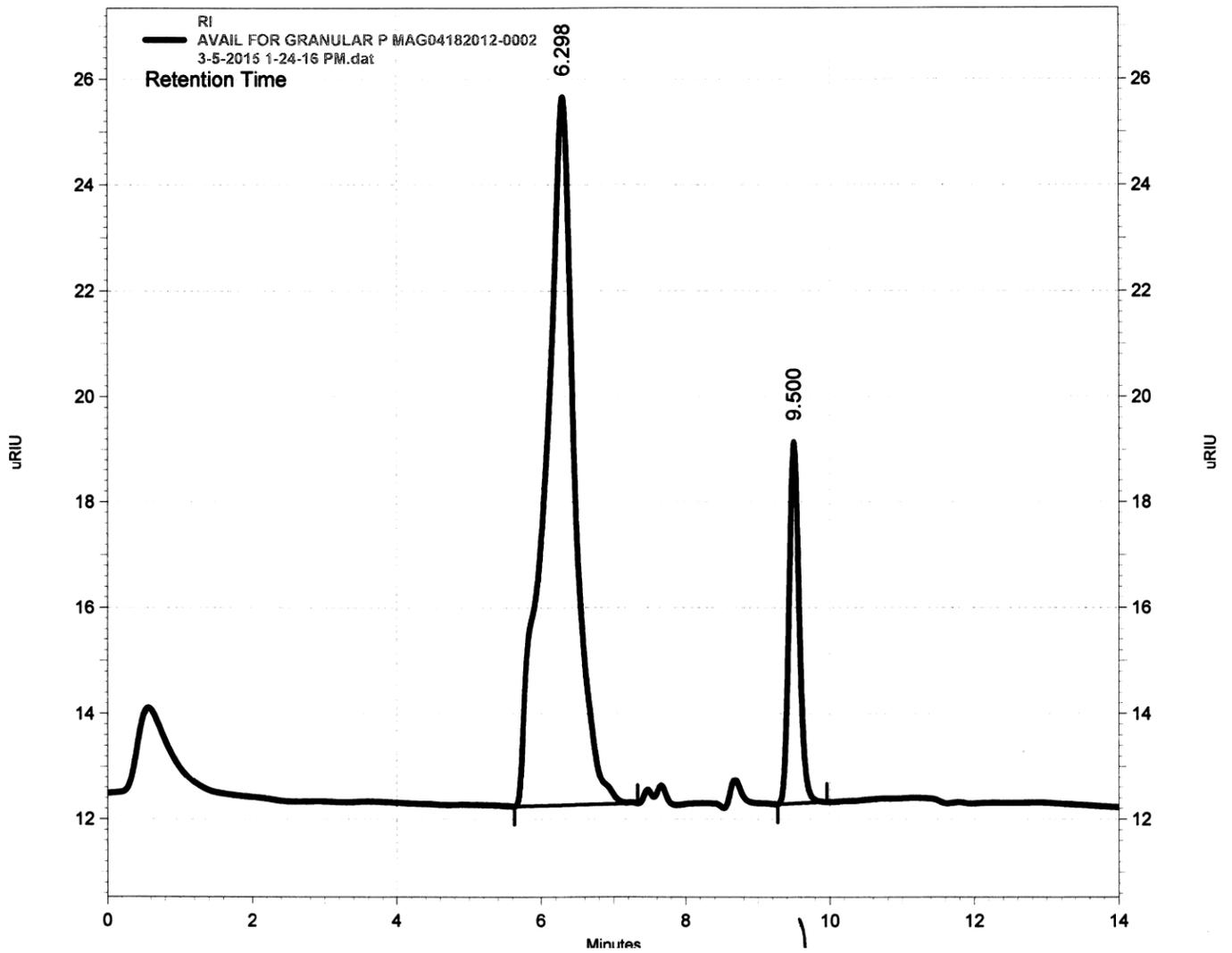
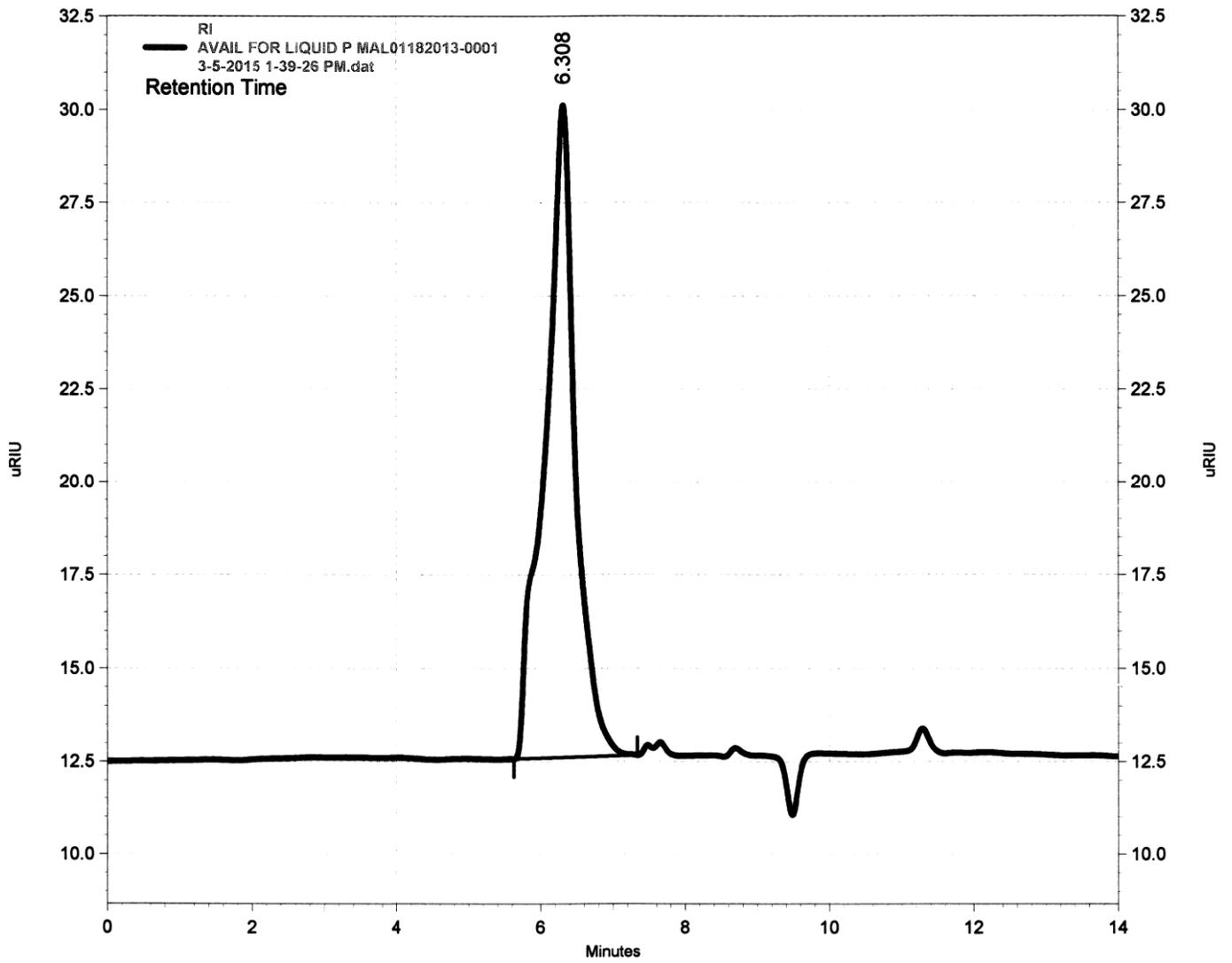


Figure 7. Avail for Liquid P Fertilizers



### Counterion Identification

The counterions declared for each Verdesian Life Sciences polymer product are listed in the table below.

Product	Counterion	Method No.
NutriSphere-N for Liquid Nitrogen Fertilizers	Calcium	AOAC 965.09 <sup>2</sup>
NutriSphere-N for Granular Nitrogen Fertilizers	Calcium	AOAC 965.09
Avail for Liquid Phosphate Fertilizers	Ammonium	APHA/AWWA Method 4500-NH <sub>3</sub> D <sup>3</sup>
Avail for Granular Phosphate Fertilizers	Sodium	AOAC 983.04 <sup>4</sup>

NutriSphere-N for liquid nitrogen fertilizers is tested for calcium content per AOAC method 965.09; test results indicating calcium levels above 50 ppm give confirmation of counter ion identity

NutriSphere-N for granular nitrogen fertilizers is tested for calcium content per AOAC method 965.09; test results indicating calcium levels above 75 ppm give confirmation of counter ion identity

Avail for granular phosphate fertilizers is tested for sodium content per AOAC method 983.04; test results indicating sodium levels above 100 ppm give confirmation of counter ion identity.

Avail for liquid phosphate fertilizers is tested for ammonium content per APHA/AWWA method 4500-NH<sub>3</sub>D; test results indicating ammonium levels above 50 ppm give confirmation of counter ion identity.

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<sup>2</sup> AOAC Official Method 965.09 Nutrients (Minor) in Fertilizers- Atomic Absorption Spectrophotometric Method

<sup>3</sup> Standard Methods for the Examination of Water and Waste Water Method 4500-NH<sub>3</sub>

<sup>4</sup> AOAC Official Method 983.04 Sodium in Fertilizers Atomic Absorption Spectrophotometric Method

### **Quantification of Polymer Components**

The percent solids content of NutriSphere-N for Liquid Nitrogen Fertilizers, Avail for Liquid Phosphate Fertilizers, and Avail for Granular Phosphate Fertilizers, are determined by subtraction of the water value as determined by Karl Fischer titration (ASTM E203-08<sup>5</sup>) from 100% to obtain the percent solids content for each product. The solids content is equal to the percentage of itaconic maleic copolymer salt claimed on the product label.

In the case of NutriSphere-N for Granular Nitrogen Fertilizers, the both propylene glycol content as determined by SEC chromatography, and the water content as determined by Karl Fischer titration (ASTM E203-08) are subtracted from 100% to obtain the percent solids content for this product. The solids content is equal to the percentage of itaconic maleic copolymer salt claimed on the product label.

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<sup>5</sup> ASTM E203-8 Standard Test Method of Water Using Volumetric Karl Fischer Titration