Response to Questions Raised by Tom Phillips Concerning the Enforcement Analyitical Method for Avail and Nutrisphere Products

Verdesain Life Sciences

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Philip Davidson of Maryland read a number of questions raised by Tom Phillips concerning the enforcement analyitical method for Avail and Nutrisphere products at the August 2018 AAPFCO meeting in Fort Lauderdale. The list of questions is contained in the email reproduced below and point by point responses are provided in the text that follows.

From: Tom Phillips -MDA-
To: Gary Orr
Subject: Responses to Nurtisphere and Avail products
Date: Monday, August 06, 2018 5:45:15 PM

Gary,

Here is what I sent Phil.

"Enforcement Analytical Methodology for NutriSphere and Avail Polymeric Fertilizer Enhancement Products"

1. Using an RI detector, there is no specificity in the analysis, i.e., how do we now that the polymer on the label is there?
2. The analysis is based upon physical characteristics of the polymer, i.e., molecular weight and size.
3. Would an ELSD detector been a better choice than an RI detector?
4. In reviewing the chromatography, there are multiple components in the analyte of interest. This would indicate there is a molecular weight distribution in the peak, and not a single analyte. This would make quantitation problematic giving a higher value than it actually is.
5. Propylene Glycol Quantitation Standard Chromatogram - in RI, there is always issues of valleys that form before certain types of analytes. This can be problematic in integration of the peak, and could lead to misuse of manual integration of the peaks.
6. Polyethylene Glycol - The tailing is an issue with the peak. It is not eluting completely off the column. This could cause issues with baseline shifting as the PG builds up on the column.

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

1. Which AOAC guidelines were followed? Horwitz validation, dietary supplements validation, qualitative chemistry guidelines, etc.
2. The curve in Figure 1 does not match the data in Table 1. The slope, intercept, and correlation coefficient do not match what is displayed in Figure 1.
3. The same issues, as in #2, are in Table 2, and Figure 2.
4. The same issues, as in #2, are in Table 3, and Figure 3.
5. The same issues, as in #2, are in Table 4, and Figure 4
7. If using the means of the area, then the data should be listed. It appears the tables do not contain mean data, but might be single run data.
8. The two organic acids appear to not be linear above 0.25 % (w/w)
9. Selectivity Figure 5 and 6. The resolution is not optimal. The big valley is that occurs before PG is problematic in that it can readily effect the baseline.
10. In Figure 7 and 8, there are multiple analytes in the polymer peak, as evidenced by the shoulders occurring in the peak.
11. For Figures 9 and 10, the same applies.
12. To achieve a 95% Probability of Identification, at least 90 samples are needed at varying concentration ranges. Currently, there is no way to determine if the POI/POD (Probability of Detection) is at the 95% confidence level.

Feel free to contact us if you have any questions.

Enforcement Analytical Methodology for NutriSphere and Avail Polymeric Fertilizer Enhancement Products

1.Using an RI detector, there is no specificity in the analysis, i.e., how do we know that the polymer on the label is there?

The specificity here is achieved by way of chromatography, as opposed to by means of detector. Now, the argument would be that many highly charged anionic polymers of this molecular weight range may not be differentiated from each other by this method; that is true. However, it's highly unlikely that any other highly charged anionic polymers of specific molecular weight range would be present. They are not normally present in fertilizers. More on specificity and selectivity of this method below.

2. The analysis is based upon physical characteristics of the polymer, i.e., molecular weight and size.

Strictly speaking, what we are looking at for polymers here is their retention time range under conditions described in method. This is a function of their molecular structure and, while it's influenced by their molecular weight (or, more strictly, apparent hydrodynamic volume of molecules under a strictly defined set of conditions in a defined aqueous solvent), there are additional factors involved. One of them is the chemical structure of repeat units involved, and this has a major effect on retention time of polymers; thus the analytical method actually does have a significant (though difficult to quantify) amount of selectivity between polymers varied structures at the same molecular weight. To give a sense of how selective the method is, retention time will be significantly affected by as little as 5 degrees C difference in column temperature. So we are really looking at a combination of both physical and chemical characteristics of the polymer; however the selectivity is embedded in the chromatography/columns part of the method, as opposed to the detector, but the chemical selectivity itself is definitely there. Of course, this is not as nice as a convenient LC/MS method with definitive mass spectrum of analyte as output, but it is still quite good and quite selective.

3. Would an ELSD detector been a better choice than an RI detector?

No, due to ELSD technique not being compatible with the mobile phase necessary to achieve proper chromatography of the polymer, and due to ELSD being similarly non-selective with respect to the output for analyte(s) of interest.

The scientific reason is that ELSD is not compatible with the mobile phase we use for this polymer's analysis. More generally, Waters' recommendation for analysis of highly charged polyanionic polymers is to use a suitable bank (in terms of molecular weight range of capability) of Ultrahydrogel columns containing a suitable number of columns (we normally use 3 or 1). They further recommend that these be used, in this application, with a mobile phase that is water with 0.10 M sodium nitrate.

ELSD detectors require the absence of non-volatile salts, and the analysis (to achieve proper chromatography) requires them; thus the needs of chromatography win over the detection desires, and we have to use a detector that is usable given the required eluent. This means, in practice, RI detection.

Further, these methods were developed for use by as wide a range of labs as possible, and ELSD detectors are fairly rare, while almost every large HPLC lab is likely to have at least one RI detector around.

4. In reviewing the chromatography, there are multiple components in the analyte of interest. This would indicate there is a molecular weight distribution in the peak, and not a single analyte. This would make quantitation problematic giving a higher value than it actually is.

Molecular distribution in the polymer peak is exactly what is going on. This is a "traditional" synthetic polymer with a molecular weight distribution. The way we get around this is by monitoring peak height above baseline for the polymer in analytes at Mp (peak MW) retention time. Since batch to batch reproducibility of Mp (and entire molecular weight distribution curve) is adequate due to our strict quality control procedures, we don't have an issue with major over- or underestimation due to nearby peaks that may make integration by area problematic.

5. Propylene Glycol Quantitation Standard Chromatogram - in RI, there is always issues of valleys that form before certain types of analytes. This can be problematic in integration of the peak, and could lead to misuse of manual integration of the peaks.

Correct. This is addressed in our case by two methods. First is by having sufficient separation which is usually achieved in our method by its system suitability test ensuring the columns have adequate resolving power; we generally do not have interference at the propylene glycol retention time. Second is by properly operating the instrument, the peak can be quantitatively measured in terms of peak height above the post-elution baseline, greatly reducing any integration difficulties.

6. Polyethylene Glycol - The tailing is an issue with the peak. It is not eluting completely off the column. This could cause issues with baseline shifting as the PG builds up on the column.

PEG should be eluting completely off the column. If it's not, something is wrong with the column (or it's not operated strictly according to the method) and it needs to be replaced under warranty (or column operation needs to be done in accordance with the method). The PEG standard peak should not look perfectly gaussian when viewed with a linear time scale, since retention time and molecular weight are not in a linear relationship (it's sort of logarithmic); further, the molecular weight distribution of PEG standard may not necessarily be gaussian either. But in any case, we should not have any PEG remaining on column. Note that in SEC/GPC, larger molecular weight entities elute first, and smaller ones later (ceteris paribus), with monomer/solvent coming out last. When operated in accordance with the method, there should be zero polymer buildup (PEG or analyte) on the column; we have had column sets running multiple thousands of injections of PEG and analyte polymer, with no signs of buildup.

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

1. Which AOAC guidelines were followed? Horwitz validation, dietary supplements validation, qualitative chemistry guidelines, etc.

The AOAC guidelines that we used are the AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. This has been clarified by footnote in the revised report.

2. The curve in Figure 1 does not match the data in Table 1. The slope, intercept, and correlation coefficient do not match what is displayed in Figure 1.

Figure 1 was from an earlier draft of the report and was unfortunately not caught and corrected. This figure has been swapped with the proper Figure 1 that matches the original Table 1 in the report.

3. The same issues, as in #2, are in Table 2, and Figure 2.

Figure 2 matches the data presented in Table 2. The slope of the linear regression has been corrected to 6647683.

4. The same issues, as in #2, are in Table 3, and Figure 3.

Figure 3 matches the data presented in Table 3. The slope of the linear regression has been corrected to 5811831.

5. The same issues, as in #2, are in Table 4, and Figure 4.

Figure 4 matches the data presented in Table 4. The slope of the linear regression has been corrected to 7873457.

There was not a point #6 in the original email.

7. If using the means of the area, then the data should be listed. It appears the tables do not contain mean data, but might be single run data.

The tables have revised to include replicate data.

8. The two organic acids appear to not be linear above 0.25 % (w/w)

Per the AOAC guidelines cited above "A linear response is desirable as it simplifies the calculations but is not necessary nor should it be regarded as a required performance characteristic." We feel like the linear fit used has no detrimental effect on the study, but we could alter the fit to be weighted or nonlinear as requested.

9. Selectivity Figure 5 and 6. The resolution is not optimal. The big valley is that occurs before PG is problematic in that it can readily effect the baseline.

Note that this chromatography was done with a type of size exclusion/ gel permutation column with a refractive index detector this dip is likely a byproduct of small molecule interactions within the system and does not seem unusual.

10. In Figure 7 and 8, there are multiple analytes in the polymer peak, as evidenced by the shoulders occurring in the peak.

While this would be a valid concern for a single compound, the nature of polymers makes the shoulder a moot point as most polymers are a distribution of similarly weighted compounds.

11. For Figures 9 and 10, the same applies.

See the response to point 10.

12. To achieve a 95% Probability of Identification, at least 90 samples are needed at varying concentration ranges. Currently, there is no way to determine if the POI/POD (Probability of Detection) is at the 95% confidence level.

In response to point #12, 95% Probability of Identification is, as far as I am aware of, only applicable to validation of methods involving botanicals or botanical extracts. This should not apply to the validation in question.