

series	cap color	membrane	pore size	part #
eXtremelFV®	●	PVDF	0.2µm	85531

Expedited Vitamin C Sample Preparation Through the Use of eXtremelFV® Technology

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Abstract

At Amway, Vitamin C Analysis is routinely performed for large numbers of samples. With large batches of samples for preparation and processing, each step in sample prep becomes very costly in terms of analyst time. Thomson eXtremelFV®s reduce a multi-step filtration and vial transfer process to a single step. We compared results from traditional sample preparation employing syringes, syringe filters, and HPLC autosampler vials with the results using only the Thomson Filter Vial product.

Introduction

As a common marker for nutritional products, Vitamin C analysis is routinely performed for large numbers of samples. Large batches of samples are prepared for each instrument run. Each step in the sample prep becomes a time-limiting step, with these large batches. Which translates to a larger cost in analyst time and consumables. Any reduction in time or materials can be realized as a cost savings to the lab performing the analysis.

The final steps of sample prep require the analyst to filter the sample into an HPLC autosampler vial employing a syringe and syringe filter. This is a costly step in terms of time and materials that adds little value to the final result. Any gains made at this step of the procedure can reduce the time and cost associated with Vitamin C analysis. Autosampler vials with an integrated PVDF filter are now available. The use of these vials in place of the current procedure allows several samples to be filtered at one time, reducing the time required to complete this step. The vials are also cheaper than buying a syringe, filter, and vial separately, resulting in a material cost savings in addition to the time reduction.

Method

Materials

- Thomson eXtremelFV® (0.20µm PVDF, p/n 85531)
- Thomson 48 Position Toggle Press
- Laboratory glassware and pipettes
- Extraction Solution: 1.2% HPO₃ with 10% Methanol
- DHAA Reduction Solution: 5mM TCEP, pH=2.5
- HPLC Column: Agilent Zorbax SB-Aq, 5µm, 4.6x 150mm

Instrument Method

- Isocratic 0.1% ortho-Phosphoric Acid
- Run-Time: 10 minutes
- Flow Rate: 0.6 mL/minute
- Agilent HPLC with PDA detection
- Wave Length: 245nm

Sample Preparation

Step 1

1. Samples are weighed into round bottom flasks.
2. Extraction solution is added to the flasks
3. Sample flasks with extraction solution are weighed again.



Step 2

1. Chloroform is added to flask remove fats from solution.
2. Samples are then stirred for half an hour for extraction.
3. Depending on solution thickness, samples may be centrifuged to separate



Step 3

1. 100 µL of sample is pipette into 0.2 µm filter auto sampler vial. Then 400 µL of extraction solution is added. Total volume 0.5 mL.
2. The vials are capped with filter caps and then placed in the vial press plate.
3. Once all samples have been capped they are pressed and filtered simultaneously. Once complete they are ready for analysis.



Step 4

1. All samples are run on HPLC instrument with a set method for analysis.
2. Traditional sample prep method samples were diluted and centrifuged in 15 mL centrifuge tubes and then were filtered through syringe filters into auto sampler vials.
3. Samples were then capped and injected following a sequence on the HPLC.



Results

Table 1 depicts a single sample processed using the original method, syringe and syringe filter, compared to the same sample diluted and filtrated using Thomson Filter Vials. Data for the two filtration methods were tested for equivalence using TOST. Analysis was performed using the rtost function of the equivalence package. For this test, samples are tested against the null hypothesis that the mean value for the filtration methods are different. Using a sigma value of 0.05 and epsilon corresponding to a 5% difference between the means gives a p-value = 0.00272. At this p-value, we conclude that the sample means are equivalent.

Based on the statistical testing, we have found there is no significant difference between the two filtration methods – syringe with filter and the Thomson Filter vial.

Table 1. Syringe Filtration compared to Filter Vial

Sample Filtration	Syringe w/ Filter	Thomson Filter Vial
Rep 112	67.92 69.81	68.92 68.20
Rep 314	69.93 70.31	70.01 70.79
Rep 516	69.57 70.30	70.41 71.15
Mean	69.64	69.91
Std. Dev.	0.89	1.14
% RSD	1.28	1.63

Conclusion

- No significant difference was found in the sample results between the two filtration methods.
- The Thomson eXtremIFV® can be used in place of traditional syringe and filter technique to save time and cost associated with sample preparation.

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