

Fast and Easy Sample Cleanup Solution for the Analysis of Water-Soluble Vitamins in Food Matrices

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Vitamins analysis is crucial for the labeling of vitamin-fortified food products. While this testing is a requisite for food manufacturers, it poses a challenge for them as food matrices are notoriously known for being complex, often containing high levels of undesired interferences. To make things worse, different vitamins have different chemical properties, thereby further complicating the sample cleanup process. This application brief describes a simple yet effective method of cleaning up instant noodles for the analysis of water-soluble vitamins using a quick pass-through solid phase extraction (SPE) technique.

Benefits

A simple and effective, one step pass-through SPE protocol is developed for the analysis of water-soluble vitamins in instant noodles samples.

Introduction

Vitamins are nutrients that play a vital role in maintaining the overall health and well-being of humans and animals. They may occur naturally in foods like milk and fruits or added to fortified foods like grains and cereal. Any deficiencies in intake may result in adverse health effects. Because of its importance in general health, food manufacturers are tasked to verify the vitamin label claims made on their food products. Microbiological assays and liquid chromatography (LC) are the more common techniques used in determining vitamin levels in food. They each come with pros and cons, but LC-based methods have the added advantages of being fast, accurate, and able to identify various forms of vitamins in a single run.¹ Consequently, traditional reference methods from AOAC or USP are continuously striving to modernize their vitamins analysis methods to incorporate LC or LC-MS/MS based solutions.^{2,3,4,5,6}

While LC or LC-MS/MS based methods are extremely useful, moderate level of cleanup is needed prior to sample injection to reduce sample complexity for improved data quality, increase column lifetime and maximize system uptime. Where sample cleanup is concerned, SPE is one such technique that is known to provide higher levels of cleanliness compared to its popular counterparts like protein precipitation and liquid-liquid extraction. However, one drawback of SPE is that it typically involves more method development than the aforementioned techniques. In this analysis of water-soluble vitamins in instant noodles, we attempt to use a simple and fast

past-through protocol with SPE to cleanup this protein and fat laden matrix. The pass-through procedure minimizes the extent of method development performed, whilst still providing an effective and fast sample cleanup. Oasis PRiME HLB Cartridge contains a sorbent chemistry that helps remove common food matrix interferences like fats and phospholipids.

Results and Discussion

Water-soluble vitamins are found in fortified wheat flour, the main ingredient in instant noodles. To assess the performance of the Oasis PRiME HLB Cartridge for this application, it is first important to check for the recoveries of the vitamins with this cartridge in the absence of matrix. To do this, vitamin B1, B3, B6, B12, and C standards are prepared in solution, methanol added and passed-through the Oasis PRiME HLB Cartridge. Recoveries are calculated from pre- and post-spiked peak responses. Figure 1 illustrates the chromatogram of vitamins that were present in the eluate at the end of the pass-through protocol. Comparing pre- and post-spike results, recoveries of the said water-soluble vitamins are listed in Table 1. Generally, most of the water-soluble vitamins tested gave recoveries >80% with this pass-through protocol using Oasis PRiME HLB. Vitamin C has a recovery of <50%, probably due to its stability in solution.

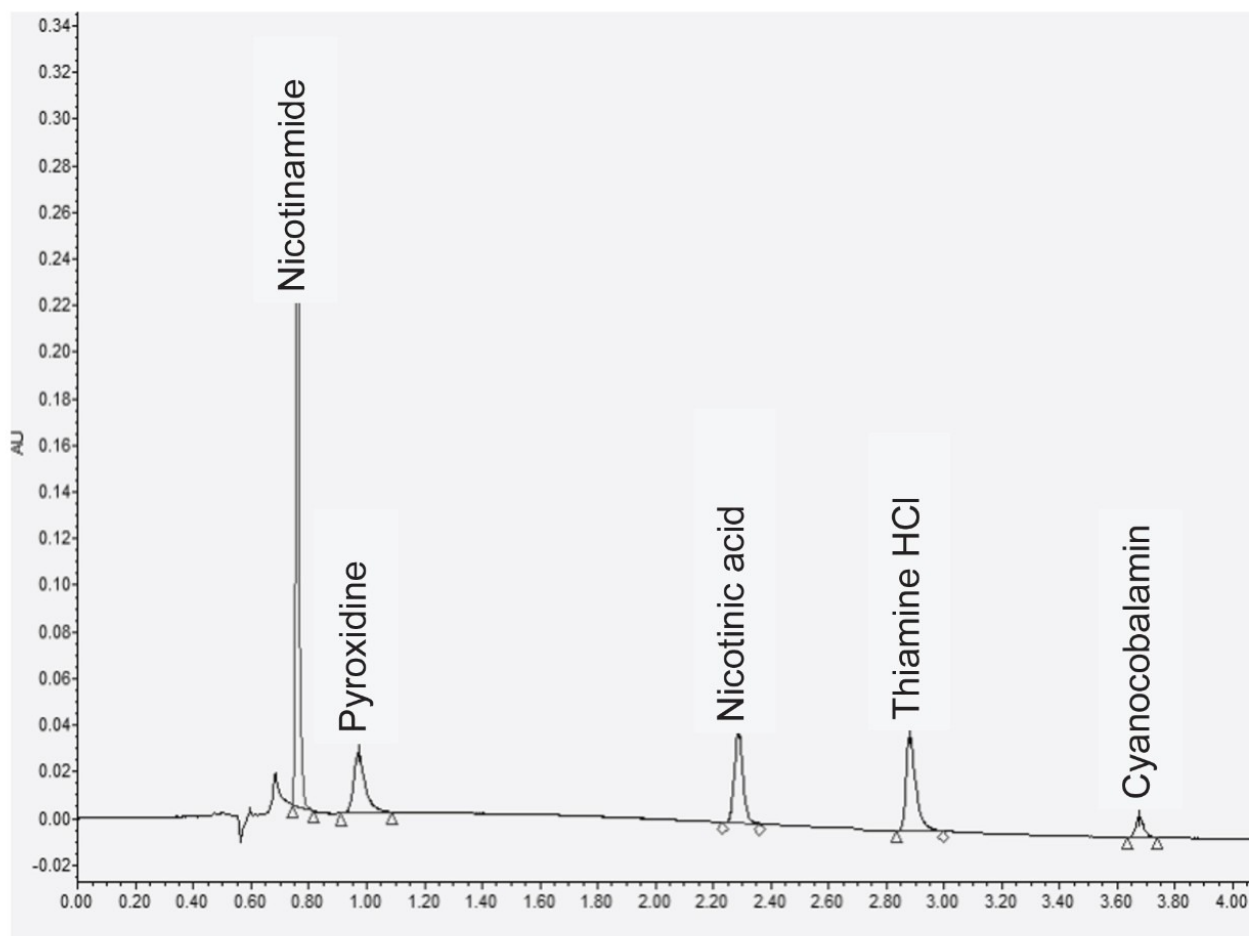


Figure 1. Chromatogram of water-soluble vitamins in solution after pass-through SPE using Oasis PRiME HLB 3cc, 60 mg ([186008056](#)). They are separated via HILIC chromatography on a 1.7 μ m ACQUITY UPLC BEH Amide Column. Peaks in order of retention times: Nicotinamide (B3), Pyroxidine (B6), Nicotinic acid (B3), Thiamine HCl (B1), Cyanocobalamin (B12).

Vitamins	Recovery (%)
Nicotinamide	85
Pyroxidine	87
Nicotinic acid	86
Thiamine HCl	87
Cyanocobalamin	85

Table 1. Recoveries.

With good recoveries obtained on standard solutions, we proceeded to assess the detection of fortified vitamins in instant noodles samples using the same pass-through SPE protocol. Briefly, vitamins were extracted from homogenized instant noodles and methanol added to the extract to precipitate out the proteins present within the sample. During the extraction step, a layer of fat was observed floating above the supernatant, which is an indication of the fat present in fried instant noodles matrix. Following precipitation, the suspension was centrifuged and the supernatant removed and loaded in its entirety into the Oasis PRiME HLB Cartridge where fat and other matrix interferences are removed as shown in Figure 2, while the eluate was collected for analysis. Figure 3 shows the detection of vitamins B1 and B6 in blank instant noodles. Total recoveries for vitamins B1 and B6 in instant noodles matrix was >80%.

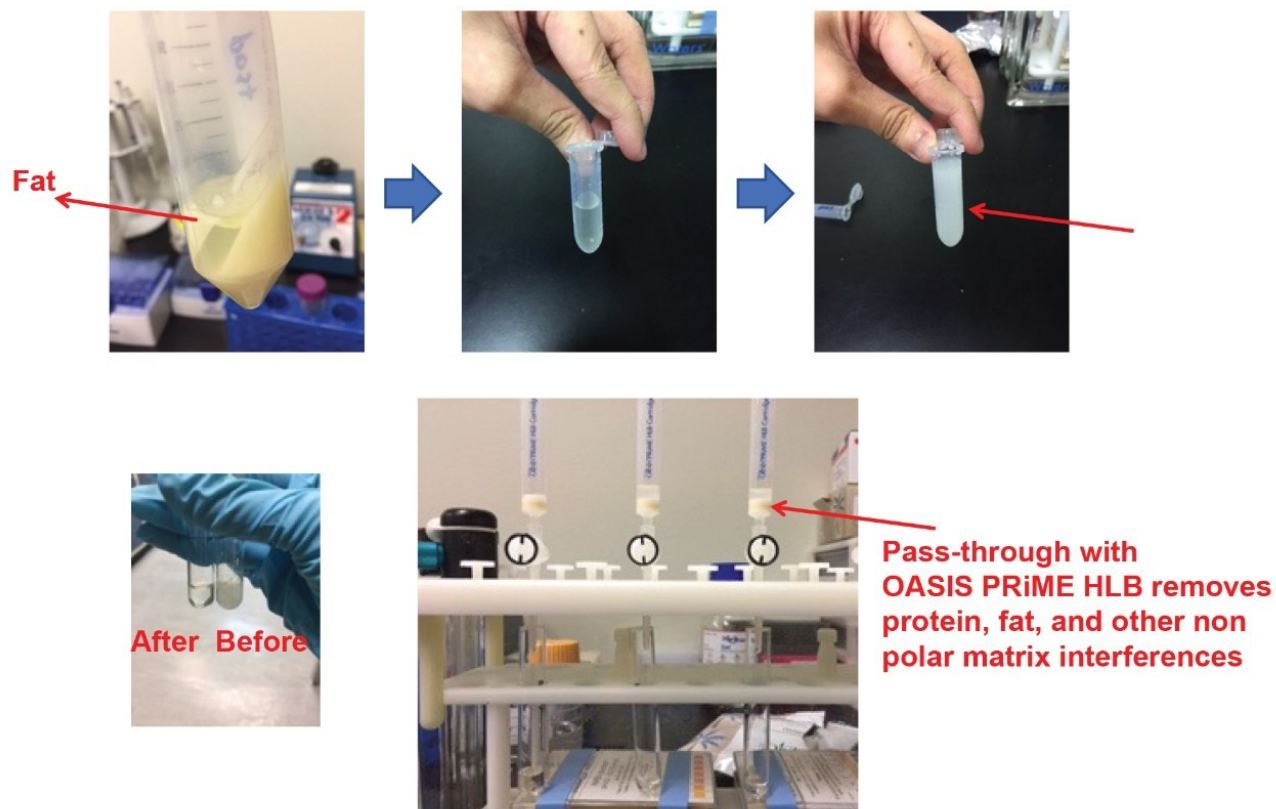


Figure 2. Pass-through procedure of cleaning up instant noodles. Homogenized noodles are extracted, and methanol added to the supernatant layer. The resulting suspension is loaded onto an Oasis PRiME HLB Cartridge and the flow-through is collected. The sample before and after SPE cleanup is shown at the bottom left image.

Homogenize instant noodles with a blender, until powdery. Weigh 2 g sample into each falcon tube.



Add 10 mL extraction solvent (0.1 M HCl), extract for 1 hour with regular shaking/vortexing at room temperature.



Centrifuge sample at full speed. To 0.5 mL supernatant, add 0.5 mL MeOH.

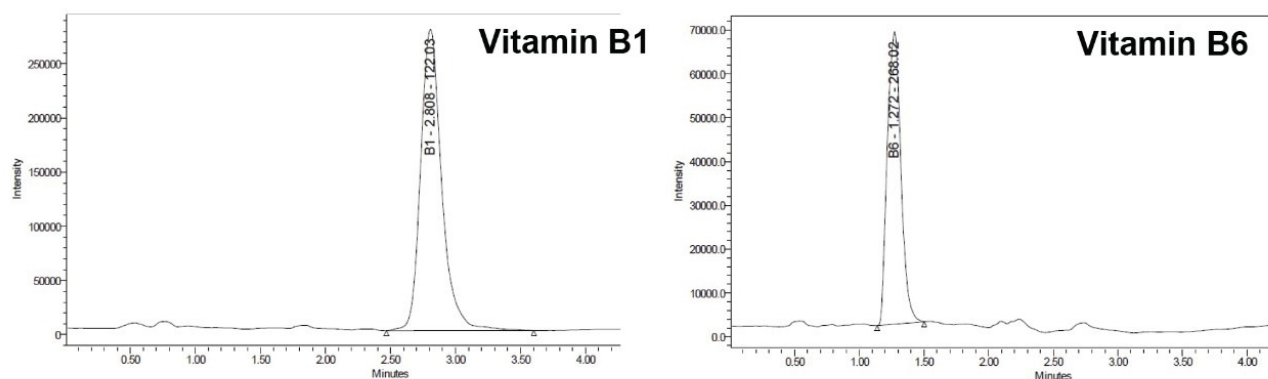


Centrifuge sample at full speed. Remove all supernatant and pass-through Oasis PRiME HLB.



Collect eluate for LC or LCMS analysis.

Flow chart of the sample preparation procedure.



**Extracted from 2 g homogenized instant noodles.*

Figure 3. Vitamins B1 and B6 detected in blank instant noodles matrix using the SPE procedure described. The ACQUITY QDa Mass Detector was used for detection.

Conclusion

A simple pass-through SPE method using Oasis PRiME HLB has been developed for the cleanup of water-soluble vitamins in instant noodles. This quick and easy protocol results in more than 80% recoveries for general B vitamins. This may be replicated in other matrices that are high in fat and proteins, as the Oasis PRiME HLB cartridge serves well to remove these interferences.

References

1. Eitenmiller, R.R. (1990), Strengths and Weaknesses of Assessing Vitamin Content Of Foods. Journal of Food Quality, 13: 7–20. <https://doi.org/10.1111/j.1745-4557.1990.tb00002.x> <<https://doi.org/10.1111/j.1745-4557.1990.tb00002.x>>.
2. J AOAC Int. Mar-Apr 2012;95(2):301–6. doi: 10.5740/jaoacint.cs2011_07.
3. J AOAC Int. 2012 Mar-Apr;95(2):322–8. doi: 10.5740/jaoacint.cs2011_15.
4. J AOAC Int. 2012 Mar-Apr;95(2):307–12. doi: 10.5740/jaoacint.cs2011_08.
5. J AOAC Int. 2016 Jan-Feb;99(1):15–8. doi: 10.5740/jaoacint.15-0250. Epub 2016 Jan 27.
6. J AOAC Int. 2016 Jan-Feb;99(1):19–25. doi: 10.5740/jaoacint.15-0245.

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