Analysis of trans-Vitamin K1 in Soy Flour and Canola Seed

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Introduction

Vitamin K1 (phyloquinone) is a fat soluble vitamin found in nature from various plant sources. It functions as a coenzyme in the body and is involved in the synthesis of several proteins involved in blood clotting and bone metabolism.1 Although synthetic vitamin K1 can consist of both the cis and trans isomer, natural vitamin K1 is almost entirely the trans isomer with only the trans isomer being biologically active.2 The main source of phyloquinone for the diet is found from green, leafy vegetables.1 Vegetable oils (rapeseed, soybean, and olive oils) are second to green, leafy vegetables as a source of vitamin K1 for the human diet.1 The knowledge that low erucic acid rapeseed oil is a major source for vitamin K1 in the human diet requires the ability to accurately test the concentration of the vitamin in these types of matrices.

Methods and Materials

The availability of methods to determine the levels of trans-Vitamin K1 in canola is limited. Due to the new OECD recommendation to test the level of vitamin K1 in canola, the initial method evaluated was AOAC Method 999.15, a method used to test infant formula and milk for total vitamin K1.3 However, it was soon determined utilizing fortified recoveries that approximately half of the total value of trans-Vitamin K1 was being extracted using AOAC Method 999.15. The attention was then turned to the USP method utilizing HPLC conditions outlined in Figure 1.

Test Materials

USP Reference Standard, Phytonadione (Vitamin K1)

Method

Approximately 1 gram of sample was weighed into a 50 mL polycrylonal conical tube, where approximately 10 mL of dimethyl sulfoxide and approximately 15 mL of hexane were added. The sample was then placed in a shaking water bath for approximately 45 minutes at 60 ± 5°C. After allowing the sample to cool, the extraction proceeded with the following steps:

1. Centrifuge (~3000rpm) ~ 10 minutes.
2. Transfer the top layer (hexane) to a 100-mL volumetric flask with a disposable transfer pipette.
3. Add 15 mL of hexane to the dimethyl sulfoxide layer in the tube, shake ~5 minutes on horizontal shaker; centrifuge (~3000rpm) for ~10 minutes.
4. Transfer the top layer to the volumetric flask.
5. Repeat Steps 3-4 one additional time.
6. Bring the 100-mL volumetric to volume with hexane.
7. Transfer 10-mL from the 100-mL volumetric to a culture tube, evaporate under nitrogen.
8. Add 1 mL of 30% DCM/MeOH Solution to the culture tube of dried residue, vortex.
9. Filter the residue into an injection vial for HPLC analysis.

Results

The method was first validated using soy flour. Figure 2 shows the 20 replicates that were completed over three days with two analysts to demonstrate precision in soy flour. Two fortified recoveries were completed at approximately 50% of the mean (0.438 µg/g) with the results of 93.8% and 90.4% to demonstrate acceptable accuracy of this method.

The scope of the assay as well as the validation was then broadened to include canola seed. Figure 3 shows the 24 replicates that were completed over three days with two analysts to demonstrate precision in canola seed. Two fortified recoveries were completed at approximately 100% of the mean (0.467 µg/g) with the results of 91.0% and 91.8% to demonstrate acceptable accuracy of this method.

Figure 4 and Figure 5 show results from the extraction efficiency of soy flour and canola seed, respectively. The results show that three extractions are not only more efficient, but that further extractions provide no additional benefit. The extraction efficiency refers to Step 5 of the Method Section. The data generated in Figures 2 and 3 utilized five extractions, however, after reviewing the extraction efficiency data, subsequent data can be generated using only three extractions.

Conclusion

Reverse phase high pressure liquid chromatography (HPLC) with post column reduction and fluorescence detection was applied to separate the inactive cis-isomer from the active form, trans-Vitamin K1, in soy flour and canola seed. Samples were extracted using organic solvents and then injected on a C30 column where trans-Vitamin K1 was quantified against standards of a known concentration. The data demonstrates acceptable precision and accuracy for extracting trans-Vitamin K1 from both canola seed and soy flour and the efficiency can be increased by performing three extraction verses the original five.

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References

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