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Question. What is the vitamin K content in various foods and supplements?

Problem. Vitamin K is very important in the body due to its effects on blood coagulation and bone metabolism, and its preventative abilities of arteriosclerosis and osteoporosis. It occurs naturally in many derivative forms, and each derivative is found abundantly in different types of food. The importance of vitamin K to the body has led to an interest in analyzing which foods and supplements contain the most vitamin K.

Proposed Solution. Similar research conducted to determine levels of other vitamins, like vitamin D, used the column switching high-performance liquid chromatography (HPLC) method. This same process was used to determine vitamin K content due to its simplicity compared to other methods, and its highly reproducible results. The column-switching component allows for the elimination of impurities and the concentration of the analytes of interest, and HPLC allows for separation, detection, and quantification of the analytes.

Technique. Each food and supplement sample were minced and weighed, and a various amount of n-hexane was added for food samples, and methanol was added for supplement samples. The solutions were shaken at 250 rpm for 5 mins and centrifuged at 2500 rpm for 5 mins. The methanol and n-hexane layers were collected. For the supplement samples, the same process above was repeated with the addition of n-hexane to the methanol layer. The n-hexane extracts were then concentrated using a centrifugal concentrator, and ethanol was added to dissolve the pellet. The samples were stored at -30°C until HPLC analysis. Column-switching HPLC was then performed on each sample.

The Requirements. About 30 mins is required to prepare each sample for HPLC, and another 30 mins to run the sample on the instrument. This means the overall procedure takes about 1 hour per sample.

Instruments and Reagents. All HPLC chemicals were of reagent grade, and a variety of meat, fish, soy, milk, and vegetable products, as well as vitamin supplements were used. During sample preparation, a POLYTRON PT-MR2100 homogenizer, Table Top Centrifuge 4000, and VC-96N centrifugal concentrator were used. The HPLC system consisted of two Jasco PU-980 HPLC pumps and a sampling injector equipped with a 20 µL sample loop. The mobile phase consisted of a 60:40 ratio of methanol and 2-propanol, with flow rates of 0.5 mL/min from each pump. The fluorescence detector was set to excitation at 320 nm and emission at 430 m. The switching valve changed positions at 3.5 mins and changed back to the starting position at 10 mins.

Precision and Limits. The RSD of the retention times for all vitamin K levels was less than 0.04%, the correlation coefficients were greater than 0.994, and the linear range was 0.879-1250 ng/mL, so vitamin K was separated with good specificity and the method

indicates good linearity. The LODs for various vitamin K concentrations ranged from 0.234-0.501 ng/mL and the LOQs ranged from 0.827-1.665 ng/mL.

Precautions. Care should be taken when working with raw meat as it may contain harmful bacteria, and surfaces should be sterilized after contact. Propanol and methanol, the mobile phase used in the HP-LC system, should also be handled with care as they are very flammable and can cause various health effects if inhaled or ingested.

Practical Applications. Although this procedure is more time consuming than procedures used for vitamin determination in the past, it has higher accuracy and specificity due to the implementation of the column-switching method, so the increase in time is accompanied by an increase in method validity. This method also allows for the quantification of each individual vitamin K derivative, rather than the quantification of vitamin K as a whole.

Search Technique. The TRU library was consulted to find articles from the journal "Food Analytical Methods." The study by Tanaka on vitamin K analysis using column-switching HP-LC included their LOD and LOQ values and proved to produce highly reproducible and quantitative results.

References.

1. Tanaka, R. *Food Analytical Methods*, **2024**, 17, 1218-1228.
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