

Examination of Vitamin K2 MK-7 Assay and Purity Determination Methods in Commercial Formulations and Matrixes

STUDY OBJECTIVES:

Vitamin K2 MK-7 is increasingly used as a consumer dietary supplement. By reason of clinically-supported bone and heart health benefits, K2 is included in mono-K2 and multi-ingredient product formulations targeting all consumer categories. Chromatographic analysis of MK-7 is a vital tool in quality determination. The widespread applicability of K2, however, promotes a broad range of product formulations/matrixes which affect chromatographic analysis.

This paper demonstrates the critical importance of MK-7 analytical method choice for consumer product testing. This investigation replicates a previously published study, using alternant methods and protocols. The objective is to demonstrate correct method choice and sample preparation can minimize inaccurate and potentially harmful conclusions. Investigation of versatile MK-7 analysis methods for different formulations/matrixes, have not previously been reported in the literature.

STUDY METHODS:

USP monograph/HPLC method(s): As of 2019 there is an official USP monograph for the analysis of Menaquinone-7⁽¹⁾, and several other chromatographic methods for the evaluation of MK-7 content. The USP monograph includes one method for quantification of MK-7, and another for *cis/trans* ratio determination. Both methods use direct solvent extraction of the vitamin from the (product form) sample.

However, in some formulations/matrixes, organic solvents alone do not extract vitamins quantitatively from the vitamin products. This is typically for products containing microencapsulated MK-7 ingredient for enhanced stability. In these cases, water and heating needs to be included in the sample preparation procedure to release the MK-7 from the matrix. This method is currently under USP submission⁽²⁾.

Microencapsulated MK-7 beadlets/HPLC method: For gelatine-based MK-7, MK-7 ingredients that use protective water-dispersible encapsulation, and consumer tablets/capsules containing these ingredient forms, a treatment with water and ethanol prior to extraction with ethyl acetate is required for correct values to be obtained. Absent this water work-up, misleading analytical results are expected, which in the context of consumer product testing can have harmful commercial consequences. It may not be apparent from the product label if a stability-enhanced ingredient was used to manufacture a consumer product.

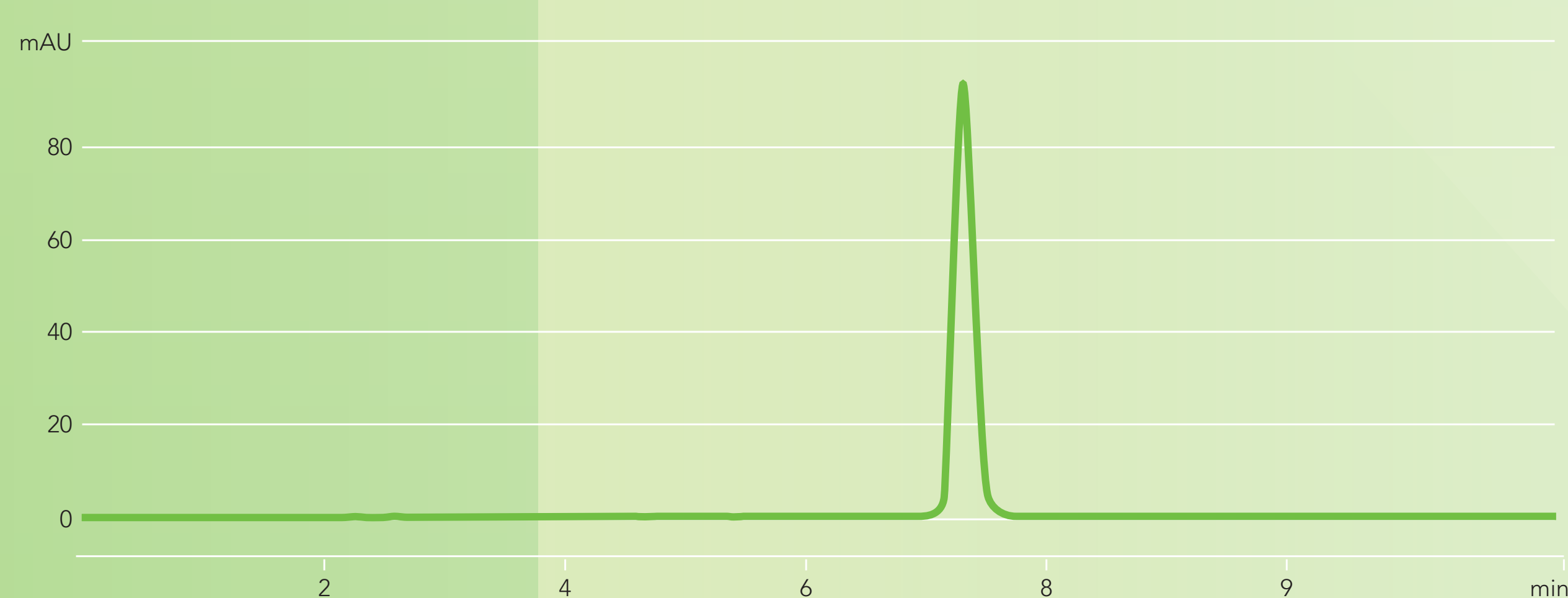


Figure 1 Chromatogram: Pure K2VITAL® K2 MK-7 Reference Standard (150 mm column)

RESEARCH DESIGN & RESULTS:

K2 MK-7 is inherently unstable in high-alkaline environments or combined with calcium or magnesium. Market studies of K2 products available to consumers globally, however, demonstrate that many consumer products use standard, unprotected K2 in these formulations⁽³⁾.

Szterk et al. recently analysed the content of MK-7 in eight consumer dietary supplements⁽⁴⁾. The authors found varying content of MK-7, concluding that five out of eight were below the declared content. The authors used tetrahydrofuran (THF) to extract MK-7 prior to analysis for all samples. Two of the sub-performing products used microencapsulated MK-7 which limits solubility to THF. Microencapsulation was not declared on the product label.

This investigation repeated the analysis of the microencapsulated products by use of the alternate microencapsulated HPLC method. Using alternate methods produced results of 102% and 105% of label claim, in contrast to published results.

Analysis Method	Molekin product Label claim 75 µg/pill	Witamina product Label claim 75 µg/pill
Amount of <i>trans</i> MK-7 presented in Szterk et al. (2) where THF was used for extraction	49.1 µg/pill	5.5 µg/pill
Amount of <i>trans</i> MK-7 by repeating extraction exactly as described in Szterk et al. where THF was used for extraction	14.0 µg/pill	10.0 µg/pill
Amount of <i>trans</i> MK-7, where the coating was dissolved in water/ethanol, with heating, prior to extraction by ethyl acetate	76 µg/pill	78 µg/pill

Table 1: The amount of detected MK-7 in microencapsulated products depends on the method of extraction

Other considerations in this investigation included MK-7 light instability and quantification of *cis*-isomers. Improper handling of the vitamin material resulting in insufficient light protection will lead to degradation of the vitamin and thus lower assay/purity results. Quantification of vitamin MK-7 *cis*-isomers in consumer products is also of importance because studies showed differences in bioavailability compared with that of all-*trans* vitamin K2 MK-7. By using only, the USP method for Menaquinone-7 content, the isomeric purity is not considered. The designed method for determination of isomeric purity should also be used to determine the all-*trans* MK-7 content. This study also investigated the isomeric purity of products.

CONCLUSIONS:

To detect MK-7 in consumer products accurately, the optimal analysis method for content and purity determination must be chosen. This re-examination of a published study demonstrates that for products containing microencapsulated MK-7, a method to dissolve coating before extracting the free vitamin from the matrix is required. Improper sample preparation procedures may lead to underestimation of the MK-7 content and inaccurate public reporting on branded products.

The presence of protective encapsulation, however, may not be declared on product packaging. Three MK-7 manufacturers offer encapsulation-protected MK-7 ingredient as of 2019). Therefore, analytical laboratories therefore must take special care in MK-7 analytical method choice and sample preparation, evaluate clues such as the presence of mineral to inform method choice, question non-intuitive results, and re-test using alternate methods when applicable. These steps reduce risk of inaccurate results and reporting that may be commercially harmful for consumer product manufacturers.

FUNDING:

None.

References

1. USP 41 – NF 36 Dietary Supplements / Menaquinone-7
2. USP-NF Pharmacopeial Forum 44(4)
3. K2 Market Study, Kappa Bioscience, self-published, 2017
4. A. Szterk, A. Zmyslowski, K. Bus, Identification of *cis/trans* isomers of menaquinone-7 in food as exemplified by food supplements, Food Chemistry 243 (2018) 403-409

Kappa Bioscience AS, based in Norway, produces Vitamin K2 as menaquinone-7 (MK-7), and developed a micro-encapsulated DELTA® product which protects the MK-7 molecule in mineral formulations and during manufacturing processes. This microencapsulation helps preserve K2 assay, and allows for novel commercial applications.



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