

Title: **Determination of water-soluble vitamins in nutritional supplements and method validation.**

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In this work a high-performance liquid chromatography (HPLC) method with reversed phase column with and photodiode array detector is described and studied in order to determine simultaneously the water-soluble vitamins present in a parenteral nutrition product from Clinical Nutrition, NutAvant Plus, a complex matrix product. This product is chosen in order to optimize the working conditions and validate the method that can later be adapted to other simpler matrix products. To optimize the method vitamins C, B₁, B₂, B₃, B₅, B₆, B₇, B₉ and B₁₂ are analysed; and different extraction methods are studied as well as the detection wavelength in the chromatographic method to improve the responses. The tests performed finally determine that vitamins B₇, B₉ and B₁₂ cannot be validated by this method.

The parameters studied for validation are: linearity, sensitivity, precision, accuracy and trueness. Linearity is measured by calibration curves and sensitivity by calculating the detection and quantization limits. Precision is determined by multiple replicates in the same day that quantifies the instrument precision and intermediate precision by multiple replicates in different days. The accuracy that is measured with an internal control material of the company. And the trueness that is calculated from fortification at different levels in the study sample.

A promising method is found that solves the problem of low concentration and chromatographic separation of the three excluded vitamins that have to be studied in the future.