

MULTIVITAMIN ANALYSIS USING LC-MS/MS

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The water-soluble vitamins are a heterogeneous group of compounds with different structures and physico-chemical properties¹ acting mainly as coenzymes. Moreover, a single vitamin consists of several biologically active forms, known as vitamers, which introduce a further element of heterogeneity due to the subtle differences in their chemical structures². For these reasons, it is very difficult to find experimental conditions suitable for their simultaneous determination, furthermore complicated by the labile nature of some vitamins, the complexity of food matrices and in the case of naturally occurring vitamins, their possible linkage with macromolecular components and by their low concentrations in foods.

Simultaneous analysis of water soluble vitamins is advantageous for several reasons such as the reduced analysis time and a cost containment due to a less extensive use of solvents, materials and equipment.

Gas-chromatography (GC) and liquid-chromatography (LC) are more suitable analytical techniques for the development of multivitamin methods; however, the selected analytes have to be visible in the selected detection system (UV detector) or have to be extractable with the same procedures from the examined food. Liquid chromatography coupled to mass spectrometry (LC-MS) has been showing itself as a promising technique in this analytical ambit. In fact, besides performing multi-analyte analysis and providing unambiguous evidence of identification, sensitivity and selectivity of LC-MS allow simplifying sample pre-treatment and restraining degradation of those vitamins susceptible to it.

Despite of LC-MS versatility, only two papers have been published about the multivitamin analysis in dietary supplements³ and in food samples⁴.

A rapid, simple and sensitive LC-ESI(+)-MS/MS method for the simultaneous determination of fourteen water-soluble vitamins (B1, B2, two B3 vitamers, B5, five B6 vitamers, B8, B9, B12 and C) in various food matrices, is here presented⁵. The use of rapid and simple extraction procedures (short exposition to light and air) and the direct injection of the extract, without any concentration step (no exposition to heat) were the two important outcomes achieved as a consequence of the high sensitivity and selectivity of LC-MS/MS. The mild extraction conditions preserved the analytes susceptible to degradation, allowed estimation of each vitamer singly and avoided artifacts formation. For these reasons, our method was able to minimize the loss of those vitamins that are particularly susceptible to degradation and to accurately characterize the profile of free forms in foodstuffs.

¹Belitz H-D, Grosch W, Schieberle P. Vitamins. In Food Chemistry, (3rd revised ed.). Springer-Verlag: Berlin Heidelberg, Germany, 2004; 409.

²Ball GFM. *Bioavailability and analysis of vitamins in foods*; London: Chapman and Hall, 1998

³Chen, P., Wolf, W.R. (2007). *Anal. Bioanal. Chem.*, 387, 2441-2448

⁴Leporati, A., Catellani, D., Suman, M., Andreoli, R., Manini, P., Niessen, W.M.A. (2005). *Anal. Chim. Acta*, 531, 87-95.

⁵Gentili, A., Caretti, F., D'Ascenzo, G., Marchese, S., Perret, D., Di Corcia, D., Mainero Rocca, L. (2008). *Rapid Commun. Mass Spectrom.*, 22, 2029-2043.