Abstract

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Measurement of intracellular vitamin C levels in human lymphocytes by reverse phase high performance liquid chromatography (HPLC).

Emadi-Konjin P, Verjee Z, Levin AV, Adeli K.

Department of Paediatrics Laboratory Medicine, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

OBJECTIVES: Vitamin C plays an active role in many important metabolic processes, such as collagen formation and the prevention of bleeding. Although overt scurvy is now rare, there is evidence that subclinical vitamin C deficiency is still quite common. Serum and plasma vitamin C measurements do not correlate well with tissue levels while lymphocyte vitamin C levels provide the most accurate assessment of the true status of vitamin C stores and are not affected acutely by circadian rhythm or dietary changes. We report a specific and reproducible reverse phase high performance liquid chromatographic method (HPLC) for the quantification of vitamin C in lymphocytes.

METHODS: Reverse phase HPLC with a UV detection system was used. National Committee for Clinical Laboratory Standards (NCCLS) guidelines were followed for evaluation. Sample stability testing for lymphocyte vitamin C was performed for a period of 24 h at room temperature and 4 degrees C. Lymphocyte vitamin C levels were measured in 51 children.

RESULTS: Lymphocyte vitamin C measurement with HPLC revealed very good analytical sensitivity with a 1.42 microg/10(8) lymphocyte lower limit of detection on repeated testing. An external standard curve was used for quantification, which showed a linear range of 1.25-100 microg/10(8) lymphocyte with a correlation coefficient of 0.989. Precision studies showed an inter-assay repeatability coefficient of variance (CV) between 0.25-9.98% and a within-assay coefficient of variance between 1.2-12.49%. The inter-assay CV for a period of 20 days was less than 10% for concentrations equal to or less than 1.42 microg/10(8) lymphocytes and less than 5.5% for concentrations between 5-100 microg/10(8) lymphocytes. Vitamin C was most stable at 4 degrees C, with a 0.31% decrease after 3 h and 2.35% after 4 h. At room temperature, vitamin C loss was more significant, with losses of 8.44% and 15.6% at 3 and 4 h, respectively, at a concentration of 29.9 microg/10(8) lymphocytes.

CONCLUSIONS: The proposed HPLC method offers a reliable and reproducible technique for the quantification of intracellular vitamin C. Lymphocyte samples can be rapidly prepared and represent a more homogeneous tissue sample source for intracellular vitamin C measurement as compared to serum. To ensure stability, lymphocyte lysates should be prepared and stored at or below -20 degrees C within 2 h of blood collection.

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