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A Comparison of Three High Performance Liquid Chromatographic (HPLC) Methods for Measurement of Plasma Total Homocysteine

Emin Özgür AKGÜL[1], Erdinç ÇAKIR[2], Ömer ÖZCAN[2], Halil YAMAN[1], Cumhur BİLGİ[2], Mehmet Kemal ERBİL[1]

[1] Department of Biochemistry, Gülhane Military Medical School, Ankara - Turkey

[2] Department of Emergency Medicine, Gülhane Military Medical School, Ankara - Turkey

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Authors

medsci@tubitak.gov.tr

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Abstract: Homocysteine (tHcy) has been in routine use in many centers after this amino acid was reported as an independent risk factor for cardiovascular, cerebrovascular and peripheral vascular diseases. Many high-performance liquid chromatographic (HPLC) methods including a commercially available kit (Chromsystems) were reported for measuring tHcy in biologic samples. Although the commercial kits are believed to have higher precision, sensitivity and accuracy as well as practical and fast, it may cause high prices by increased test ordering. In this study we aimed to compare two different low-cost methods which had similar advantages with commercial kit as a reference method. In the first method the sample was derivatized with 7-fluoro-2,1,3-benzoxa sulfonamide (ABD-F) following the reduction with tris-(2-carboxylethyl) phosphine (TCEP). Homocysteine peak was obtained at 4.7 min and total run time was 7 min. In the second method, samples were derivatized with 7-Fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid (SBD-F). Homocysteine peak was obtained at 2.9 min and total run time was 5 min. In both methods cysteamine hydrochloride was added as an internal standard and C18 (ODS) (150 X 4.6 mm, 5 µm) column was used. Intraassay CV's of SBD-F and ABD-F methods was 0.98% and 1.83%, respectively. Interassay CV values were 2.22% and 2.49%, respectively. Both methods show linearity up to 200 µmol /L. Recovery was 99.37% and 98.10% in SBD-F method after additions of 7-14 µmol / L homocysteine standard. Similarly, for ABD-F method, recovery was 99.7% and 100.7% following the addition of homocysteine standard (6.7-13.4 µmol/L). A significant correlation was found between both methods and reference method ($P < 0.001$). In conclusion, SBD-F and ABD-F methods were reproducible, rapid, and easy to use for the quantification of plasma tHcy, with similar advantages of the commercially available kit. Moreover, the SBD-F method may be the method of choice among these assays because of the lowest cost per test and better precision.

Key Words: Homocysteine, HPLC, Method, SBDF, ABDF

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