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Chromatographic method for estimation of vitamin E from dried blood spot sample

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Abstract

Background: We developed a selective bioanalytical RP-HPLC method for estimation of vitamin E from dried blood spot (DBS) sample, a potential technique which can be used for population-based epidemiological studies. Vitamin E was extracted from DBS by using liquid-liquid extraction technique with methanol (100% v/v) as reconstituting solvent for the residue. Alpha tocopheryl acetate was used as internal standard. Samples were analyzed directly on HPLC with C_{18} (250 × 4.6 mm × 5 μm) Phenomenex column. The mobile phase used was methanol to water (99:1% v/v) at a flow rate of 1.4 mL/min. The detector wavelength used was 292 nm.

Results: The retention time observed for vitamin E and internal standard was 10.225 ± 0.00075 min and 13.580 ± 0.00075 min respectively. The vitamin E calibration curve was found to be linear over the range of 0.625 to 60 μg/mL. The limit of quantification for vitamin E was found to be 0.1 μg/mL. Accuracy of the developed method was found to be 103.179%, 101.625%, and 100.174% with percentage of coefficient of variation of 0.0161, 0.0215, and 0.2790 for HQC, MQC, and LQC samples respectively which were within USFDA acceptance limit of ± 15 to ± 20%.The intraday and interday precision expressed as coefficient of variation were 0.0191–0.0841% and 0.0074–0.0252% respectively.

Conclusions: The method represents a simple, rapid, specific, accurate, and precise method for estimation of vitamin E in human blood using DBS technique. The developed method can be further evaluated with respect to effect of matrix variability before it can be used in clinical setting.

Keywords: Vitamin E, RP-HPLC, Dry blood spot (DBS), Alpha tocopheryl acetate