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Box–Behnken Design Based Development of UV-Reversed Phase High Performance Liquid Chromatographic Method for Determination of Ascorbic Acid in Tablet Formulations

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Abstract

A simple, sensitive, accurate and inexpensive UV-reversed-phase high-performance liquid chromatographic method was developed for the determination of ascorbic acid in tablet formulations. The method was based on the separation of ascorbic acid using a mobile phase of an acetonitrile-NaH_2PO_4-H_3PO_4 buffer solution (pH = 3) (5:95 v/v) with a UV detection wavelength of 245 nm and a flow rate of 0.8 mL min^{-1} at ambient column temperature. The variables of the proposed method, such as acetonitrile fraction (%), flow rate (mL min^{-1}) and column temperature (°C), were optimized on the peak area by response surface methodology via the Box–Behnken design. The mobile phase was passed isocratically, and the separation of ascorbic acid was performed at the retention time of 4.1 min. A calibration graph was obtained and found to be linear in the concentration range of 10–180 μg mL^{-1}. The method suitability was assessed and an asymmetry factor of 1.15 was obtained. The proposed method was successfully applied for the determination of ascorbic acid in tablet formulations and statistically compared with the results of the reference method. The performance of the proposed method was excellent and in agreement with the reference method. The recovery percentage of the proposed and reference methods was in the range of 99.98–100.04% and showed compliance (100 ± 2%) with regulatory guidelines. © 2022 by the authors.

Author keywords

ascorbic acid; Box–Behnken design; HPLC; ICH; validation

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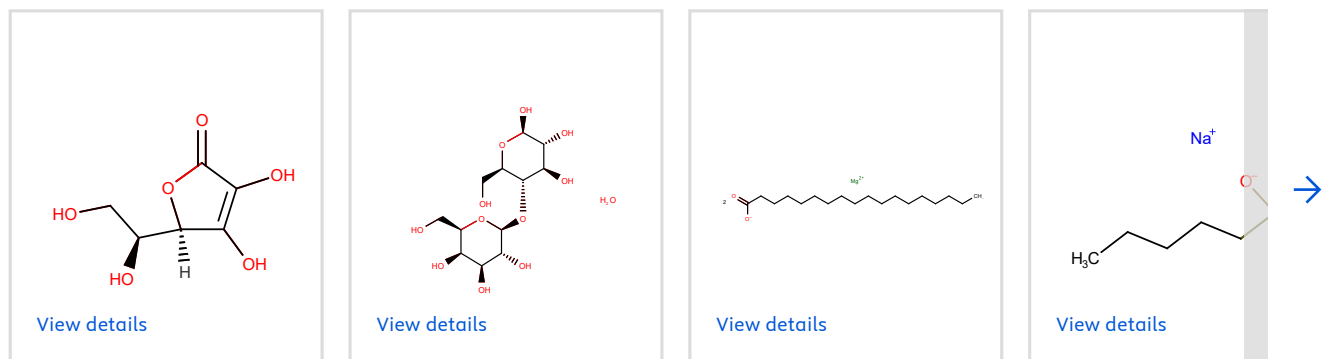
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