IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, July 2024

A Comprehensive Review on Analytical Method Development and Validation Using High Performance Liquid Chromatography (HPLC)

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Abstract: *Chromatography, while fundamentally a separation technique, is extensively utilized in chemical analysis, with High-Performance Liquid Chromatography (HPLC) being one of the most versatile methods available. In HPLC, analytes are separated by passing them through a column packed with micrometer-sized particles. Among the various HPLC techniques, reversed-phase chromatography (RP-HPLC) has become the most commonly used. This popularity stems from its simplicity, versatility, and ability to handle compounds of diverse polarity and molecular mass.*

Reversed-phase chromatography is particularly valued in both analytical and preparative applications within biochemical separation and purification. It is especially effective for molecules with hydrophobic characteristics, such as proteins, peptides, and nucleic acids, offering excellent recovery and resolution.This review highlights the critical role of RP-HPLC in analytical method development, exploring strategies for optimizing chromatographic parameters to achieve efficient method development. Key parameters, such as mobile phase composition, column selection, flow rate, temperature, and detection wavelength, are discussed to provide a comprehensive understanding of the process. The review aims to elucidate the significance of RP-HPLC in developing robust and reliable analytical methods, ensuring accurate and precise chemical analysis.

Keywords: HPLC, RP-HPLC, Analytical methods, Chromatographic parameter

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DOI: 10.48175/568