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Short Communication

Role of Reverse-Phase Chromatography Coupled with Mass Spectrometry

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ABOUT THE STUDY

Reverse-Phase Chromatography (RFC) is a widely used analytical technique in chemistry and biochemistry. It is a form of liquid chromatography that relies on the principles of hydrophobic interactions between molecules and a stationary phase to separate and analyze compounds in a mixture. Reverse-phase chromatography operates on the principle that nonpolar molecules or compounds with hydrophobic properties tend to interact more strongly with a nonpolar stationary phase than polar molecules [1,2].

This interaction causes the compounds to be retained and separated based on their hydrophobicity. Reverse-phase chromatography relies on the principle of hydrophobic interactions between the analyte molecules and the nonpolar stationary phase. The more hydrophobic a compound is longer it will be retained on the column. It is used in diverse applications, such as pharmaceuticals, environmental analysis, food chemistry, and proteomics. Researchers can prepare the stationary phase and mobile phase to suit the specific compounds they want to separate. While reverse-phase chromatography is highly versatile, it may not be suitable for compounds with extreme hydrophobicity or hydrophilicity [3-6].

The stationary phase in reverse-phase chromatography is typically composed of hydrophobic materials like C18-bonded silica. It is especially useful in drug discovery and quality control, where precise separation and quantification of active pharmaceutical ingredients are crucial. Analysts can run reverse-phase chromatography methods by adjusting parameters such as column chemistry, mobile phase composition, flow rate, and temperature [7-10].

Reverse-phase chromatography is frequently coupled with Mass Spectrometry (LC-MS) for qualitative and quantitative analysis. This technique is compatible with a variety of sample matrices, including biological fluids (e.g., blood, urine, and serum) and complex mixtures. Sample preparation is often straightforward, and the method can be adapted to accommodate a wide range of sample types. The mobile phase in reverse-phase chromatography is typically a polar solvent, often a mixture of water and organic solvents like acetonitrile or methanol. It is commonly used for the for the separation and analysis of drugs, peptides, proteins, nucleic acids, and small organic molecules [11-13].

Reverse-phase chromatography offers high sensitivity and selectivity, making it an excellent choice for quantifying and identifying compounds in complex mixtures. The choice of the column (i.e., particle size, length, and chemistry) is crucial in reverse-phase chromatography. It can significantly impact the resolution and separation efficiency of the analytes.

Reverse-phase chromatography is frequently coupled with Mass Spectrometry (LC-MS) for compound identification. This combination allows for not only separation but also precise determination of molecular weights and structural information [14].

Over the years, there have been significant advancements in column technology, detector sensitivity, and software for data analysis in reverse-phase chromatography, improving its overall performance. Advancements in column materials, particle sizes, and instrumentation have improved the efficiency and speed of reverse-phase chromatography, making it even more accessible and powerful for researchers. Despite its widespread use, reverse-phase chromatography has some limitations [15].

CONCLUSION

In conclusion, reverse-phase chromatography is a versatile and potent technique for the separation and analysis of a wide range of compounds. Its ability to provide high sensitivity, selectivity, and compatibility with mass spectrometry has made it an indispensable tool in various scientific disciplines. Researchers continue to explore and refine its applications, ensuring its relevance in modern analytical chemistry. Scientists continue to refine and innovate this technique to address new challenges and applications in the ever-evolving field of analytical chemistry.

REFERENCES

1. Herrero M, Ibanez E, Cifuentes A, Bernal J. Multidimensional chromatography in food analysis. J Chromatogr A. 2009;1216(43): 7110-29.

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2. Guttman A, Varoglu M, Khandurina J. Multidimensional separations in the pharmaceutical arena. Drug Discov Today. 2004;9(3):136-44.
3. Vailaya A, Horváth C. Retention in reversed-phase chromatography: partition or adsorption. J Chromatogr A. 1998;829(1-2):1-27.
4. Johansson K, Frederiksen SS, Degerman M, Breil MP, Mollerup JM, Nilsson B, et al. Combined effects of potassium chloride and ethanol as mobile phase modulators on hydrophobic interaction and reversed-phase chromatography of three insulin variants. J Chromatogr A. 2015;1381:64-73.
5. Melander W, Horváth C. Salt effects on hydrophobic interactions in precipitation and chromatography of proteins: an interpretation of the lyotropic series. Arch Biochem Biophys. 1977;183(1):200-15.
6. Chomet M, Van Dongen GA, Vugts DJ. State of the art in radiolabeling of antibodies with common and uncommon radiometals for preclinical and clinical immuno-PET. Bioconjug Chem. 2021;32(7):1315-30.
7. Morais M, Ma MT. Site-specific chelator-antibody conjugation for PET and SPECT imaging with radiometals. Drug Discov. Today Technol. 2018;30:91-104.
8. Studzińska S, Rola R, Buszewski B. The impact of ion-pairing reagents on the selectivity and sensitivity in the analysis of modified oligonucleotides in serum samples by liquid chromatography coupled with tandem mass spectrometry. J Pharm Biomed Anal. 2017;138:146-52.
9. Murugaiah V, Zedalis W, Lavine G, Charisse K, Manoharan M. Reversed-phase high-performance liquid chromatography method for simultaneous analysis of two liposome-formulated short interfering RNA duplexes. Anal Biochem. 2010;401(1):61-7.
10. Wei B, Goyon A, Zhang K. Analysis of therapeutic nucleic acids by capillary electrophoresis. J. Pharm Biomed Anal. 2022:114928.
11. Goyon A, Yehl P, Zhang K. Characterization of therapeutic oligonucleotides by liquid chromatography. J Pharm Biomed Anal. 2020;182:113105.
12. Willemse CM, Stander MA, Vestner J, Tredoux AG, De Villiers A. Comprehensive two-dimensional hydrophilic interaction chromatography (HILIC)× reversed-phase liquid chromatography coupled to high-resolution mass spectrometry (RP-LC-UV-MS) analysis of anthocyanins and derived pigments in red wine. Anal Chem. 2015;87(24):12006-15.
13. Ding K, Xu Y, Wang H, Duan C, Guan Y. A vacuum assisted dynamic evaporation interface for two-dimensional normal phase/reverse phase liquid chromatography. J Chromatogr A. 2010;1217(34):5477-83.
14. Atapattu SN. Solvation properties of acetone-water mobile phases in reversed-phase liquid chromatography. J Chromatogr A. 2021;1650:462252.
15. Qiao X, Zhang L, Zhang N, Wang X, Qin X, Yan H, et.al. Imidazolium embedded C8 based stationary phase for simultaneous reversed-phase/hydrophilic interaction mixed-mode chromatography. J Chromatogr A. 2015;1400:107-16.