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Efficient enantiomer separation techniques for vitamins; short review

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Abstract

The role of the review is to discuss how to separate enantiomers of vitamins. Pharmaceutical, nutraceutical, and agrochemical industries are particularly emphasized in this article. High-performance liquid Chromatography (HPLC) is a widely used technique because it is versatile and efficient for separation. HPLC uses chiral stationary phases such as cyclodextrins, polysaccharides, and protein-based chiral stationary phases to resolve vitamins such as E, D, and C. Capillary electrophoresis (CE) can separate chiral vitamins such as B6 and B12 by utilizing electric field-induced differential enantiomer migration. This is accomplished through 'chiral selectors', compounds that can only bind selectively to one enantiomer, thereby aiding in their separation process. Standard chiral selectors include crown ethers and cyclodextrins. Gas Chromatography (GC), less frequently employed for vitamins than other techniques, works well provided columns have coatings consisting of chiral stationary phases like cyclodextrins or polysaccharides. The latter has been proved in the resolution of the enantiomers of vitamins A and K, suggesting that it could be applied more in future times. Supercritical Fluid Chromatography (SFC) is an eco-friendly way to separate vitamin B1 and B5 enantiomers. It employs supercritical fluids as the mobile phase. This approach can utilize different chiral stationary phases and quickly analyze samples while consuming less solvent, making it environmentally friendly. Enantiomeric separation methodologies guarantee the quality and efficacy of products in the pharmaceutical and nutraceutical industries. Continuous advancement in chiral analysis and optimum chemical usage is needed to maintain high product quality and efficacy standards. This highlights the need for more rigorous research on enantiomer separation techniques.

Keywords: Enantiomers', separation, vitamins, SFC, technique.

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Introduction

Enantiomers in vitamins

Enantiomers are molecules that do not have the same shape when viewed upon reflection in a mirror. This means their mirror images cannot be superimposed on each other, even though they share the same chemical formula[1]. In vitamins, enantiomer's biological activities and pharmacological effects significantly differ. Separation and isolation of enantiomers are essential in manufacturing nutraceuticals[2]. This is important for guaranteeing the efficacy and safety of vitamin formulations. Herein, we explore advanced techniques to efficiently separate vitamin-related enantiomers, including traditional and modern methodologies, together with illustrative case studies and applications[3]. The goal is to underscore the importance of separating enantiomers in producing vitamins, thereby advancing quality control and targeted therapeutics.

Enantiomers separation in vitamins

Different enantiomers in our body show different behaviour, which is critical for vitamins[4]. Vitamins are crucial since most exist in chiral forms with two enantiomers. Sometimes, one might be helpful, while the other can have no effect or harm[5]. Therefore, separating the enantiomers to deliver proper form with enormous possibilities for wellness benefits becomes necessary.

Traditional methods for separating enantiomers in vitamins

Methods for resolution

One of the earliest methods used for enantiomer separation is called resolution[6]. The technique involves utilizing enantiomers' varying solubilities or reaction rates with specific chiral compounds, such as resolving agents or enzymes[7]. By utilizing these differences, the enantiomers can be separated and obtained in their forms[8], as shown in Fig (1).

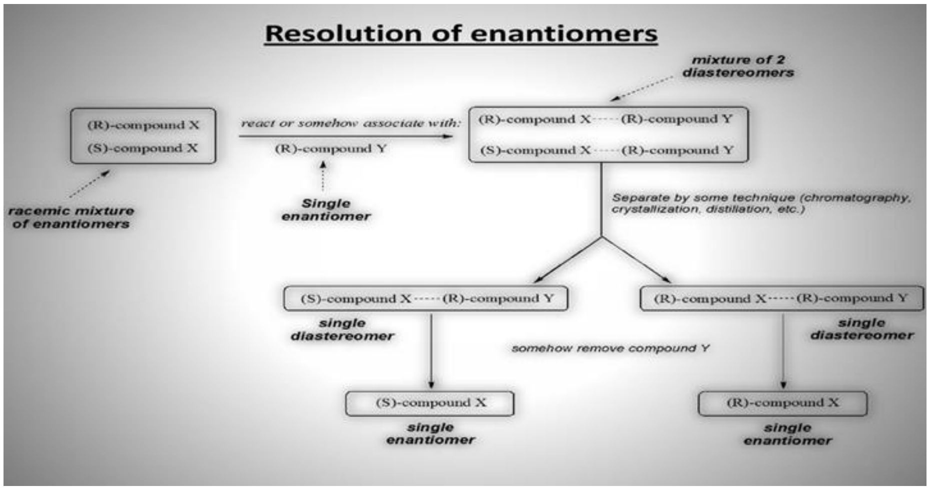

Figure (1). Resolution of enantiomers.[8]

Crystallization techniques

Enantiomer separation has also been accomplished using crystallization techniques[9]. It is feasible to produce distinct crystals with the desired enantiomer by establishing circumstances that favour the selective crystallization of one enantiomer over the other[10], as illustrated in Fig. 2

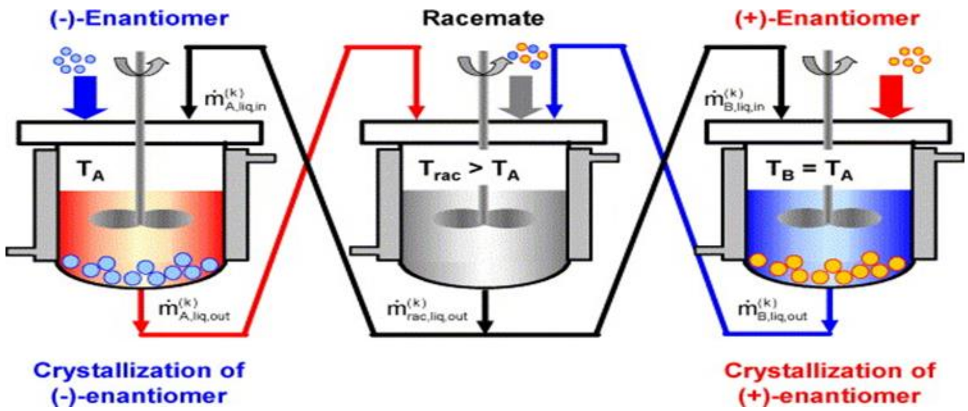

Figure (2). An arrangement designed for achieving a quasi-continuous, simultaneous production of both enantiomers within a conglomerate through preferential crystallization (Lorenz, et al., 2006).

Derivatization approaches

Another commonly utilized approach for separating enantiomers is derivatization. This process involves modifying one of the enantiomers by adding a chiral molecule, known as a derivatizing agent. This alteration enables the separation of enantiomers based on their unique properties, such as boiling point or chromatographic behavior[11-13].

Enantiomer separation techniques using chromatographic methods

One of the powerful approaches for separating enantiomers is HPLC, which works on a stationary phase of chiral selectors. This allows different interactions with the enantiomers, a fantastic scientific process that makes them separate[14]. HPLC is known for its excellent resolution, and it is highly used in pharmaceutical and research laboratories[15].

High performance (HPLC) of Liquid chromatography

HPLC is a strong chromatographic technique for separating enantiomers[16], which involves the chiral selector as the stationary phase; an enjoyable scientific process that makes them[17]. It has exceptionally high resolution and is thus very useful in pharmaceutical and research laboratories[18]. For instance, the study of the separation of water-soluble vitamins on FO-SiO_2. Column and aminopropylated silica expand these methods, primarily demonstrating through the work that a silica column functionalized with fluorine, FO-SiO_2, makes a unique difference in the separation of water-soluble vitamins[19]. The study provides a thorough comparison of separation by FO-SiO_2 and aminopropylated silica, showing that the former greatly enhances separation efficiency and is quite forgiving in the gradient used. This is related to the distinct way in which the various vitamin molecules interact with the column material under the two different conditions. [20].

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In contrast, the aminopropylated silica column contains amino groups, which provide extra separation mechanisms. These are particularly helpful for separating vitamins that vary in polarity and solubility[21]. Using a dual-column system, the authors accomplished a not very far removed, stripped-down version of the HPLC of water-soluble vitamins[22]. Moreover, this system separates the vitamins, eliminating the undesired contaminants. This work represents an essential step for the authors and water-soluble vitamin HPLC because it is both an ingenious idea and a well-executed application of the precision and power of advanced separation methods using silica-based materials[23].

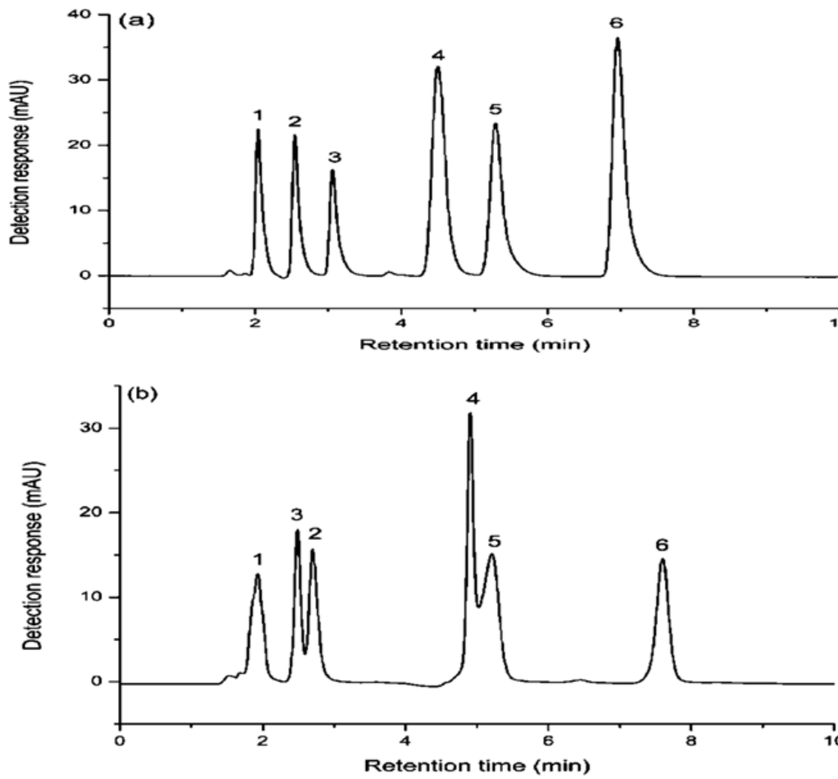

Figure (3) Water-soluble vitamin separation using FOSiO2-column and aminopropylated silica (Liu et al., 2014).

Gas chromatography (GC)

Gas chromatography (GC) is a chromatographic method for enantiomer separation[24]. In GC, enantiomers are volatilized and separated based on their different affinities for the stationary phase or the chiral selector[25]. Gas chromatography (GC) has significant utility in the separation of volatile compounds, making it valuable in applications such as environmental and food analysis[26], as illustrated in Fig. (4).

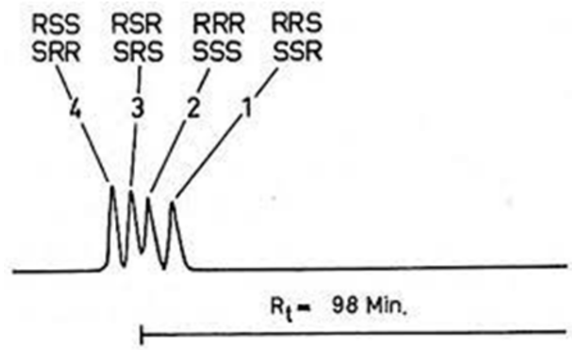

Figure (4) The chromatographic representation of all rac-α-Toc-ME (vitamin E), all four sets of diastereomers of all-rac-α-Toc-ME show consistent peak heights, Fu et al., 2017).

Supercritical fluid chromatography (SFC)

Supercritical fluid chromatography (SFC) is a technique that combines the properties of both liquid and gas chromatography to separate enantiomers[27]. Supercritical fluid chromatography (SFC) utilizes a mobile phase supercritical fluid, predominantly carbon dioxide, and a chiral stationary phase[28]. This technique, known for its speed and efficiency, is one of the leading methods for enantiomer separation. Its versatility in separating chiral compounds quickly and effectively has made it a valuable tool for scientists and analysts in diverse fields, including pharmaceutical analysis, food analysis, and environmental monitoring. This method is visually represented in Fig. 5,[29].

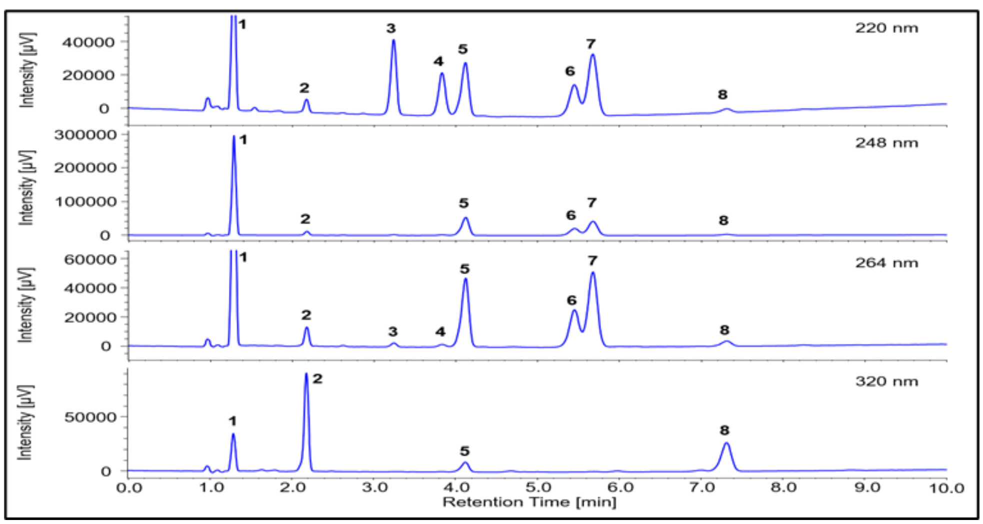

Figure (5). Chromatograms of standard solution (1: Vitamin K3, 2: Vitamin A acetate, 3: Vitamin E acetate, 4: Vitamin E, 5: Vitamin K1, 6: Vitamin D2, 7: Vitamin D3, 8: Vitamin A palmitate) (JASCO Global., 2023).

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Exploring the latest separation advances in the enantiomers of vitamins

Enantioselective membrane processes

In this context, enantioselective membrane processes have garnered significant interest as a promising approach to enantiomer separation[30]. This process, which relies on a meticulously designed membrane with chiral recognition ability, selectively facilitates the transport of the enantiomer across the membrane, as depicted in Fig. 6[31]. The potential for large-scale separation applications is particularly exciting, hinting at a promising future for enantioselective membrane processes[32].

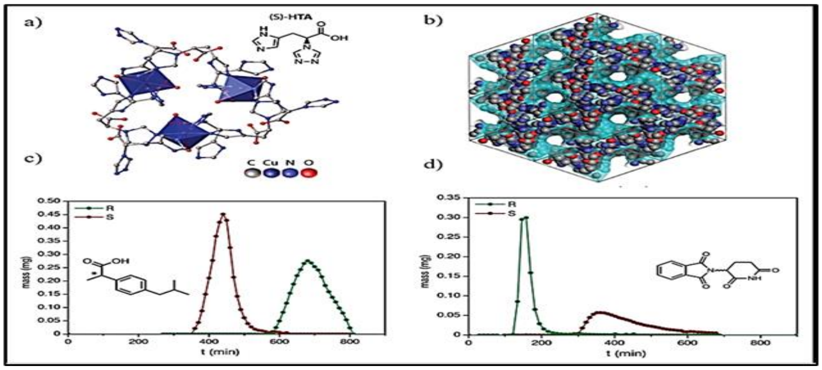

Figure (6). Metal-Organic Frameworks Enantioselective Separations.[32]

Molecular Imprinting Techniques

One such technique is molecular imprinting, where specific cavities or'molds' of a particular enantiomer are created in a polymer matrix for selective binding and separation[33]. It is a flexible tool that could be modulated to meet different requirements for enantiomer separation[34]. Conversely, MIPs have many advantages, like predictable structure, chemical stability, and specificity in molecular recognition[35]. What truly inspires the authors is the remarkable versatility of MIPs in various applications, from drug delivery to environmental remediation. The generation process for MIPs involves a three-step process: pre-polymerisation, polymerisation, and subsequent removal of the template, as shown in Figure 7.[36]

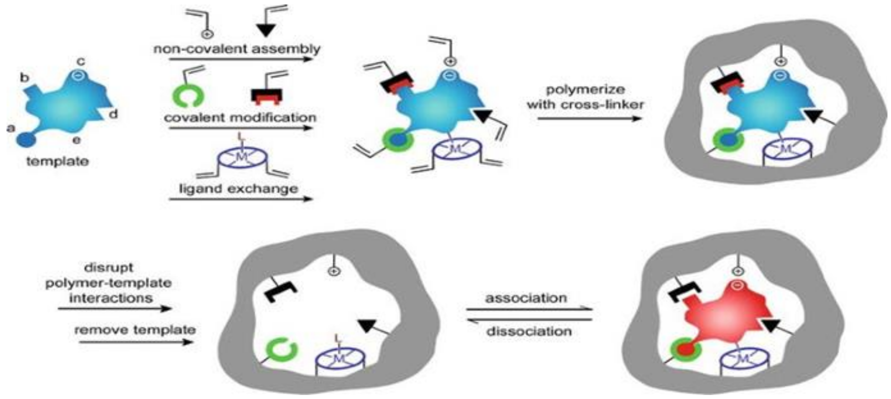

Figure (7). A demonstrated figure to describe the process of construction of the MIP.[36]

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Chiral ligand-exchange chromatography

Chiral ligand-exchange chromatography is a cutting-edge technique that combines chiral chromatography principles with ligand exchange[37]. This method uses metal ions as chiral selectors, creating complexes with enantiomers to facilitate their separation[38]. This method is particularly effective in separating difficult-to-separate enantiomers and holds great promise for researchers and scientists seeking to ensure the highest quality of vitamins for maximum health benefits[39-41].

Enantiomer innovative separation techniques

Techniques for the separation of vitamins for enantiomers are also continuously developing to cope with the urgent and increasing demand for pure enantiomers[42]. This pressing need keeps scientists and researchers on their toes, continuously seeking out new developments that would increase the speed and effectiveness of these techniques, such as developing new chiral stationary phases, improvements in enantioselective synthesis methods, and advances in resolution techniques[43].

Challenges of enantiomer separation

With the improved techniques for separating enantiomers, some challenges still need to be considered[44]. One major challenge is the cost of their separation techniques; some methods are expensive and consume a lot of time[45]. Issues of scalability for mass production exist with these techniques regarding vitamins. However, the potential impact of ongoing research on developing more cost-effective and scalable separation techniques is inspiring[46].

Prospects

The future of enantiomer separation techniques within the vitamin domain is bright, with further research and development work expected to lead to more efficient and cost-effective methods. The potential impact of these advancements, such as artificial intelligence and automation, is significant and could inspire new ways of thinking and working in the industry. These developments would further enable the production of high-quality vitamins with enhanced therapeutic capabilities, motivating the audience to continue their essential work.

Conclusions

In connection with this, the efficacy of the techniques of enantiomer separation assumes a very significant dimension in vitamin manufacture. Chiral stationary phases, enantioselective synthesis, and resolution techniques have been developed to isolate pure enantiomers of the vitamins. This makes it very relevant and applicable that efficient enantiomer separation in the production of vitamins is essential to the following analysis of case studies and applications. Although specific challenges exist, the potential of these techniques does look promising with the continuous innovations and developments that are going on in this field. The role of practical techniques for separating enantiomers is crucial, as it ensures the vitamins' purity, potency, and safety, providing reassurance about the quality of the products.

Moreover, methods like resolution and crystallization have led to the development of chromatographic separation methods and enantioselective synthesis. In addition, they utilize chiral stationary phases, while new techniques like molecular imprinting show some outstanding potential for the

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future. However, scalability and cost-related issues are yet to be resolved. It is through further improvements in enantiomer separation that quality and value in vitamin formulations can be bettered toward achieving improved health outcomes for people worldwide. The importance of continuous exploration and refinement in this field cannot be overstated, as it is the key to achieving our goals and improving health outcomes.

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Conflict of Interest

There is no known conflict associated with this work.

Author's Contribution Statement

Rana A Hamza: contributed to the study's conception

Abeer K Shams: conducted a literature review, designed the figures, conducted

Ekhals A. Salman: Drafted the initial manuscript.

Khalid Zainulabdeen: Editing and reviewing the literature.

Noor M. Ali, Safaa Mohamed, Nany Hairunisa , Amra Bratovcic , Amamer Redwan, and Emad Yousif: Editing and supervision.

References

1. Izadyari M, Naseem MT, Müstecaplıoğlu ÖE. Enantiomer detection via quantum Otto cycle. *Phys Rev E*. 2023;107(4). doi:10.1103/physreve.107.l042103.
2. Lucci E, et al. Enantioselective high-performance liquid chromatographic separations to study occurrence and fate of chiral pesticides in soil, water, and agricultural products. *J Chromatogr A*. 2022;1685:463595. doi:10.1016/j.chroma.2022.463595.
3. Chavan VD, et al. Advanced functional materials and their coordinated composites for next-generation Li-S batteries: A brief review. *J Energy Storage*. 2024;88:111572. doi:10.1016/j.est.2024.111572.
4. Chen W, et al. Fabrication and application of chiral separation membranes: A review. *Sep Purif Technol*. 2023;327:124898. doi:10.1016/j.seppur.2023.124898.
5. Alvarez-Rivera G, Bueno M, Ballesteros-Vivas D, Cifuentes A. Chiral analysis in food science. *TrAC Trends Anal Chem*. 2020;123:115761. doi:10.1016/j.trac.2019.115761.
6. Santos R, Pontes KV, Nogueira IBR. Enantiomers and their resolution. *Encyclopedia*. 2022;2(1):151-188. doi:10.3390/encyclopedia2010011.
7. Duan J, He M, Hu B. Chiral speciation and determination of selenomethionine enantiomers in selenized yeast by ligand-exchange micellar electrokinetic capillary chromatography

after solid phase extraction. *J Chromatogr A*. 2012;1268:173-179. doi:10.1016/j.chroma.2012.10.002.

8. Fu JY, Htar TT, De Silva L, Tan D, Chuah LH. Chromatographic separation of vitamin E enantiomers. *Molecules*. 2017;22(2):233. doi:10.3390/molecules22020233.
9. Han J, Soloshonok VA, Klika KD, Drabowicz J, Wzorek A. Synthesis of chiral fluorinated compounds: Methods and applications. *Chem Soc Rev*. 2018;47(4):1307-1331. doi:10.1039/c6cs00703a.
10. Hinze WL, Riehl TE, Armstrong DW, DeMond W, Alak A, Ward T. Liquid chromatographic separation of enantiomers using a chiral β-cyclodextrin-bonded stationary phase and conventional aqueous-organic mobile phases. *Anal Chem*. 1985;57(1):237-242. doi:10.1021/ac00279a055.
11. Ilisz I, Berkecz R, Péter A. Application of chiral derivatizing agents in the high-performance liquid chromatographic separation of amino acid enantiomers: A review. *J Pharm Biomed Anal*. 2008;47(1):1-15. doi:10.1016/j.jpba.2007.12.013.
12. JASCO Inc. Analysis of fat-soluble vitamins by supercritical fluid chromatography system [Internet]. 2020 [cited 2020 Nov 19]. Available from: https://www.jasco-global.com/solutions/analysis-of-fat-soluble-vitamins-by-supercritical-fluid-chromatography-system
13. Al-Sulaimi S, Kushwah R, Alsibani MA, Jery AE, Aldrdery M, Ashraf GA. Emerging developments in separation techniques and analysis of chiral pharmaceuticals. *Molecules*. 2023;28(17):6175. doi:10.3390/molecules28176175.
14. Hussen AA. High-performance liquid chromatography (HPLC): A review. *Ann Adv Chem*. 2022;6(1):10-20. doi:10.29328/journal.aac.1001026.
15. Sidana J, Joshi LK. Recycle HPLC: A powerful tool for the purification of natural products. *Chromatogr Res Int*. 2013;2013:509812. doi:10.1155/2013/509812.
16. Ibrahim AE, El Gohary NA, Aboushady D, et al. Recent advances in chiral selectors immobilization and chiral mobile phase additives in liquid chromatographic enantio-separations: A review. J Chromatogr A. 2023;1706:464214. doi:10.1016/j.chroma.2023.464214.
17. Géhin C, Holman SW. Advances in high-resolution mass spectrometry applied to pharmaceuticals in 2020: A whole new age of information. *Anal Sci Adv*. 2021;2(3-4):142-156. doi:10.1002/ansa.202000149.
18. Liu H, Guo Y, Wang X, Liang X, Liu X, Jiang S. A novel fullerene oxide functionalized silica composite as stationary phase for high-performance liquid chromatography. *RSC Adv*. 2014;4(34):17541-17548. doi:10.1039/c4ra01408a.
19. Sentkowska A, Piwowarczyk S, Pyrzyńska K. Simultaneous determination of vitamin B6 and catechins in dietary supplements by ZIC-HILIC chromatography and their antioxidant interactions. *Eur Food Res Technol*. 2020;246(8):1609-1615. doi:10.1007/s00217-020-03516-w.

20. Layne J. Characterization and comparison of the chromatographic performance of conventional, polar-embedded, and polar-endcapped reversed-phase liquid chromatography stationary phases. *J Chromatogr A*. 2002;957(2):149-164. doi:10.1016/s0021-9673(02)00193-0.
21. Rizzolo A, Polesello S. Chromatographic determination of vitamins in foods. *J Chromatogr A*. 1992;624(1-2):103-152. doi:10.1016/0021-9673(92)85676-k.
22. Mateeva A, Kondeva-Burdina M, Peikova L, Guncheva S, Zlatkov A, Georgieva M. Simultaneous analysis of water-soluble and fat-soluble vitamins through RP-HPLC/DAD in food supplements and brewer's yeast. *Heliyon*. 2023;9(1):e12706. doi:10.1016/j.heliyon.2022.e12706.
23. Xie S, Yuan L. Recent progress of chiral stationary phases for separation of enantiomers in gas chromatography. *J Sep Sci*. 2016;40(1):124-137. doi:10.1002/jssc.201600808.
24. Lehotay SJ, Hajšlová J. Application of gas chromatography in food analysis. *TrAC Trends Anal Chem*. 2002;21(9-10):686-697. doi:10.1016/s0165-9936(02)00805-1.
25. Deng H, et al. Application of chiral and achiral supercritical fluid chromatography in pesticide analysis: A review. *J Chromatogr A*. 2020;1634:461684. doi:10.1016/j.chroma.2020.461684.
26. West C. Current trends in supercritical fluid chromatography. *Anal Bioanal Chem*. 2018;410(25):6441-6457. doi:10.1007/s00216-018-1267-4.
27. Plachká K, Tesařová E, Gerlichová Z, Chocholouš P, Kubíček P. Columns in analytical-scale supercritical fluid chromatography: From traditional to unconventional chemistries. J Sep Sci. 2023;46(18). doi:10.1002/jssc.202300431
28. Analysis of Fat-soluble Vitamins by Supercritical Fluid Chromatography System | JASCO Global [Internet]. JASCO Inc.; 2020 Nov 19. Available from: https://www.jasco-global.com/solutions/analysis-of-fat-soluble-vitamins-by-supercritical-fluid-chromatography-system
29. Choi H-J, Ahn Y-H, Koh D-Y. Enantioselective mixed matrix membranes for chiral resolution. Membranes (Basel). 2021;11(4):279. doi:10.3390/membranes11040279
30. Corella-Ochoa MN, Valenzano L, Canivet J, Aguado S, Farrusseng D. Homochiral metal-organic frameworks for enantioselective separations in liquid chromatography. J Am Chem Soc. 2019;141(36):14306–16. doi:10.1021/jacs.9b06500.
31. Zhu Q, Cai Z, Zhou P, Sun X, Xu J. Recent progress of membrane technology for chiral separation: A comprehensive review. Sep Purif Technol. 2023;309:123077. doi:10.1016/j.seppur.2022.123077.
32. Gkika DA, Katsaros FK, Karasali Y, Papageorgiou EP, Giannakopoulos KN. Application of molecularly imprinted polymers (MIPs) as environmental separation tools. RSC Adv Polym Sci. 2024;2(2):127–48. doi:10.1039/d3lp00203a.

33. Sarafraz-Yazdi A, Razavi N. Application of molecularly-imprinted polymers in solid-phase microextraction techniques. TrAC Trends Anal Chem. 2015;73:81–90. doi:10.1016/j.trac.2015.05.004.
34. Sumaya AY, Risan MH, Ürker O, Yousif E. Identification, characterization and antibiotic susceptibility testing of Pseudomonas aeruginosa isolated from clinical sources. Iraqi Journal of Bioscience and Biomedical. 2024 May 8;1(1):27-37.
35. Piletsky SA, Chianella I, Whitcombe MJ. Molecularly imprinted polymers. In: Springer eBooks. 2013. p. 1596–9. doi:10.1007/978-3-642-16712-6_719.
36. Brinkman UAT, Irth H. Ligand exchange principles for trace enrichment and selective detection of ionic compounds. In: Springer eBooks. 1990. p. 195–212. doi:10.1007/978-94-009-0777-5_19.
37. Qing H, Jiang X, Yu J. Separation of tryptophan enantiomers by ligand-exchange chromatography with novel chiral ionic liquids ligand. Chirality. 2014;26(3):160–5. doi:10.1002/chir.22286
38. Orazio GD, Asensio-Ramos M, Fanali C. Enantiomers separation by capillary electrochromatography using polysaccharide-based stationary phases. J Sep Sci. 2018;42(1):360–84. doi:10.1002/jssc.201800798.
39. Mattrey FT, Baumann T, Elmasry MS, Gallegos M, Gazda R. Current challenges and future prospects in chromatographic method development for pharmaceutical research. TrAC Trends Anal Chem. 2017;95:36–46. doi:10.1016/j.trac.2017.07.021
40. Liu X, Li J, Zhang Q, Dirbeba MG. Separation of chiral enantiomers by optical force and torque induced by tightly focused vector polarized hollow beams. Phys Chem Chem Phys. 2019;21(28):15339–45. doi:10.1039/c9cp02101a
41. Wu Y, Zhang N, Luo K, Liu Y, Bai Z, Tang S. Recent advances of innovative and high-efficiency stationary phases for chromatographic separations. TrAC Trends Anal Chem. 2022;153:116647. doi:10.1016/j.trac.2022.116647
42. Su X, Zeng W, Zheng M, Jiang X, Lin W, Xu A. Big data analytics capabilities and organizational performance: The mediating effect of dual innovations. Eur J Innov Manag. 2021;25(4):1142–60. doi:10.1108/ejim-10-2020-0431
43. Mingrui Z, Xiufang S, Ninghui Y. The challenges and solutions of chiral drug preparation techniques. Deleted J. 2024. doi:10.54647/chemistry350041
44. Yaashikaa PR, Devi MK, Kumar PS. Biohydrogen production: An outlook on methods, constraints, economic analysis and future prospect. Int J Hydrogen Energy. 2022;47(98):41488–506. doi:10.1016/j.ijhydene.2022.07.082
45. Naghdi E, Ahmadloo R, Shadi M, Moran GE. Chiral purification by enantioselective extraction: Principles and recent development. Trends Environ Anal Chem. 2023;40:e00219. doi:10.1016/j.teac.2023.e00219.Berenjian A, Yazdanpanah N. Unveiling the Latest Breakthroughs in Menaquinone-7 Research through Fermentation-Based Production. Processes. 2023 Aug 30;11(9):2593.