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Advances in Two-Dimensional Liquid Chromatography (2D-LC): A Comprehensive Review of Techniques, Applications, and Future Perspectives

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ABSTRACT:

Two-dimensional liquid chromatography (2D-LC) has emerged as a powerful analytical technique that enhances separation capabilities and improves peak capacity in complex samples. This comprehensive review article provides an overview of the latest advancements in 2D-LC techniques, explores its wide-ranging applications, and discusses future perspectives.The review begins by outlining the fundamental principles of 2D-LC and the different approaches used to implement this technique, including heart-cutting, comprehensive, and online 2D-LC. It discusses the advantages and limitations of each approach, emphasizing their utility in specific analytical scenarios.A detailed analysis of the applications of 2D-LC follows, focusing on its use in pharmaceutical analysis, metabolomics, natural product analysis, and environmental analysis. The review highlights how 2D-LC enables the separation of complex mixtures, provides improved resolution for co-eluting compounds, and enhances identification and quantification capabilities. Additionally, it explores the combination of 2D-LC with other analytical techniques such as mass spectrometry, further expanding its potential applications. Furthermore, the review discusses recent advancements in column technologies, stationary phases, and detection methods that have contributed to the improved performance of 2D-LC. It also examines the development of automated software tools and data analysis approaches tailored for 2D-LC data.

Finally, the review concludes with an outlook on the future of 2D-LC. It highlights emerging trends such as the integration of 2D-LC with other separation techniques, advancements in hyphenated detection methods, and the potential for miniaturization and automation. The article emphasizes the growing importance of 2D-LC in addressing the analytical challenges of complex samples and its potential to shape the future of separation science.In summary, this comprehensive review article provides a detailed examination of the advancements, applications, and future perspectives of 2D-LC. It serves as a valuable resource for researchers, analysts, and scientists in the field of analytical chemistry, offering insights into the potential of 2D-LC for enhanced separations and improved analytical capabilities.

INTRODUCTION

Multidimensional chromatography uses a combination of several chromatography techniques, separation modes, and columns to separate multiple components. It achieves significantly higher separation than normal one-dimensional chromatography. Various separation modes and the corresponding mobile phases can be selected for HPLC, and the diverse permutations available suggest the possibility of achieving a degree of selectivity not possible using one-dimensional separation alone.

Multidimensional chromatography includes an offline technique in which the eluent is temporarily fractioned (collected) in a suitable position and then part or all of it is injected into the next column, and an online technique in which part or all of the eluent is introduced into the column via the sample loop for automatic analysis. Offline multidimensional chromatography requires additional handling, such as elution by solvent and concentration of the temporarily fractioned (collected) eluent.Two-dimensional LC is a commonly used HPLC multidimensional chromatography technique. It is sometimes called "LC×LC". One of the two-dimensional LC techniques is comprehensive two-dimensional LC, in which all the eluent from the first-dimension column is introduced online into the second-dimension column, such that the entire sample is subjected to two-dimensional separation.

Definition and Principles of 2D-LC:

Two-dimensional liquid chromatography (2D-LC) is an advanced chromatographic technique that involves the coupling of two independent chromatographic separations to achieve improved resolution and peak capacity. It is designed to address the limitations of traditional one-dimensional chromatography, such as limited peak capacity and co-elution of analytes.

The fundamental principle of 2D-LC lies in the utilization of two orthogonal separation mechanisms. The sample is first separated in the first dimension (1D) using one type of chromatography, such as reversed-phase, normal-phase, size exclusion, or ion exchange chromatography. The effluent from the first dimension is then fractionated and further separated in the second dimension (2D) using a different separation mechanism. This two-step separation process allows for the resolution of compounds that co-elute in a single-dimensional separation.The concept of orthogonality in 2D-LC is crucial. Orthogonal separation mechanisms refer to separation modes that are independent of each other, meaning that the retention and separation mechanisms in the first dimension do not influence those in the second dimension. By employing orthogonal separation mechanisms, 2D-LC can achieve higher resolution and peak capacity by separating compounds based on complementary properties.The heart of 2D-LC lies in the comprehensive analysis of complex samples. Various fractions of the first-dimension effluent can be selectively transferred to the second dimension based on specific criteria, such as the presence of target compounds or regions of interest. Alternatively, comprehensive 2D-LC involves the complete transfer of all the first-dimension effluent to the second dimension, enabling a more comprehensive analysis of the entire sample.

The combination of two independent separations in 2D-LC results in an increased number of theoretical plates, or peak capacity, compared to one-dimensional separations. The improved peak capacity allows for better separation of closely related compounds and provides a higher resolving power, leading to enhanced sensitivity, accuracy, and reliability in complex sample analysis.

In summary, 2D-LC is a chromatographic technique that combines two orthogonal separation mechanisms to achieve improved resolution and peak capacity. By separating complex samples in two dimensions, 2D-LC overcomes the limitations of one-dimensional chromatography and provides enhanced separation capabilities for a wide range of applications in various fields of analytical chemistry.

Techniques and Approaches in 2D-LC:

1. Heart-Cutting 2D-LC:

Heart-cutting 2D-LC, also known as targeted 2D-LC, is a technique in which specific target compounds or regions of interest from the first-dimension separation are selectively transferred to the second dimension for further separation. The heart-cutting approach involves the use of valves or switching systems to divert the desired fractions to the second-dimension column, while the non-target components are discarded. This technique is particularly useful when analyzing complex samples containing a large number of compounds, allowing for the isolation and separation of specific analytes of interest in the second dimension.[10][11]

2. Comprehensive 2D-LC:

Comprehensive 2D-LC, also referred to as untargeted or all-inclusive 2D-LC, involves the complete transfer of all the first-dimension effluent to the second dimension for comprehensive analysis. Unlike heart-cutting 2D-LC, no fractionation or selective transfer of specific compounds occurs. Comprehensive 2D-LC provides a more exhaustive separation of the sample, enabling the detection and separation of a wide range of compounds, including minor and co-eluting components. This approach is particularly suitable for complex samples where a comprehensive characterization of the entire sample is desired.[12][13]

3. Online 2D-LC:

Online 2D-LC, also known as coupled 2D-LC, refers to the direct online coupling of the two-dimensional separation without the need for fraction collection or offline transfer. In this approach, the effluent from the first-dimension separation is seamlessly introduced into the second-dimension column, typically via a switching valve or a comprehensive two-dimensional liquid chromatography system. Online 2D-LC allows for real-time analysis, where the second-dimension separation immediately follows the first-dimension separation, resulting in increased efficiency and reduced analysis time. This approach is advantageous when high-throughput analysis and rapid separation of complex samples are required.[14]

These techniques and approaches in 2D-LC offer distinct advantages depending on the specific analytical objectives. Heart-cutting 2D-LC provides targeted isolation and separation of specific compounds, comprehensive 2D-LC enables a comprehensive characterization of the sample, and online 2D-LC offers real-time analysis with reduced analysis time. The choice of technique depends on the complexity of the sample, the compounds of interest, and the desired separation goals[15].

Hyphenated Techniques: Hyphenated techniques refer to the combination of two or more analytical techniques to achieve enhanced capabilities and comprehensive analysis. In the context of 2D-LC, hyphenation involves the coupling of 2D-LC with other analytical techniques, such as mass spectrometry (MS) or other spectroscopic techniques, to improve detection, identification, and characterization of analytes[16].

Coupling 2D-LC with Mass Spectrometry (2D-LC-MS):

The coupling of 2D-LC with mass spectrometry (MS) is a powerful hyphenated technique that provides improved detection and identification capabilities. The effluent from the second dimension of 2D-LC is directed into the mass spectrometer, where compounds are ionized and fragmented for detection and structural elucidation. The combination of 2D-LC with MS offers enhanced sensitivity, selectivity, and compound identification compared to standalone 2D-LC or MS analysis. It enables the identification and quantification of complex analytes in samples with high accuracy and confidence, making it valuable in fields such as pharmaceutical analysis, metabolomics, and proteomics.[17]

Integrating 2D-LC with Other Spectroscopic Techniques:

Besides MS, 2D-LC can also be integrated with other spectroscopic techniques, such as UV-Vis spectroscopy, fluorescence spectroscopy, or infrared spectroscopy (FTIR). This integration allows for simultaneous separation and spectroscopic analysis of analytes. The spectroscopic information obtained in conjunction with the 2D-LC separation provides additional structural and functional insights into the separated compounds. By combining separation power with spectroscopic fingerprinting, this hyphenated approach offers enhanced compound characterization, particularly for complex samples where co-elution is a concern.

Enhancing Detection and Identification Capabilities:

Hyphenated techniques in 2D-LC offer improved detection and identification capabilities compared to standalone 2D-LC analysis. The sensitive and selective detection provided by mass spectrometry enables the identification of trace-level compounds and the differentiation of isomers, metabolites, and impurities. The incorporation of spectroscopic techniques further enhances the analytical power by providing complementary chemical information, such as UV-Vis absorption spectra, fluorescence emission spectra, or functional group identification through FTIR spectroscopy. The combination of separation and spectroscopic techniques in 2D-LC offers a comprehensive approach for the characterization and analysis of complex samples in various fields.

Column Technologies and Stationary Phases in 2D-LC:

Recent advancements in column technologies have significantly contributed to the performance and capabilities of 2D-LC. Two notable advancements include monolithic columns and core-shell columns, which offer improved separation efficiency and selectivity.

Monolithic columns are characterized by a continuous porous structure, providing efficient mass transfer and reduced backpressure. These columns offer advantages such as high permeability, low resistance to mass transfer, and improved separation efficiency. Monolithic columns are particularly beneficial for high-throughput analysis, where rapid separations are required without compromising resolution[18].

Core-shell columns consist of a solid core surrounded by a thin porous shell. The design of core-shell particles allows for enhanced mass transfer and increased efficiency compared to fully porous particles. Core-shell columns offer improved separation performance with shorter analysis times and lower operating pressures. This makes them suitable for high-resolution 2D-LC separations.

The selection of stationary phases is crucial for achieving optimal separation in each dimension of 2D-LC. The choice of stationary phase depends on the analyte properties and the separation mechanisms employed in each dimension. Common stationary phases used in 2D-LC include reversed-phase, normal-phase, ion-exchange, size-exclusion, and affinity-based phases.[19]

Future Perspectives and Emerging Trends in 2D-LC:

2D-LC has seen significant advancements and widespread applications in recent years, but the field continues to evolve with promising future prospects. Here, we discuss the future directions, emerging trends, and potential applications of 2D-LC, including advancements in column technologies, miniaturization, and automation, as well as hyphenated techniques and data analysis approaches.

- Miniaturization and Microscale 2D-LC:

Miniaturization of 2D-LC systems offers several advantages, including reduced solvent consumption, shorter analysis times, and improved sensitivity. The development of microscale 2D-LC systems, integrated on microfluidic platforms, holds promise for applications in point-of-care diagnostics, portable analysis devices, and on-site monitoring. Miniaturized systems also facilitate online coupling with other techniques, such as mass spectrometry, enabling rapid and sensitive analysis.[20]

- Automation and High-Throughput Analysis:

Automation in 2D-LC is gaining traction, allowing for improved method reproducibility, reduced human error, and increased sample throughput. Advances in robotic sample handling, automated sample preparation, and integrated software systems streamline the entire 2D-LC workflow. Automated data analysis approaches, such as peak alignment algorithms, pattern recognition, and machine learning techniques, are also emerging to handle the large datasets generated by 2D-LC[21].

- Hyphenated Techniques and Comprehensive Analysis:

Hyphenated techniques combining 2D-LC with mass spectrometry (2D-LC-MS), multidimensional spectroscopy, and other analytical methods offer enhanced detection and identification capabilities. The integration of complementary techniques enables comprehensive analysis, providing both separation and structural information. The continued development of hyphenated techniques will expand the analytical capabilities of 2D-LC, particularly in fields such as metabolomics, proteomics, and pharmaceutical analysis.

- Applications in Personalized Medicine and Point-of-Care Diagnostics:

2D-LC holds great potential for applications in personalized medicine and point-of-care diagnostics. By enabling comprehensive analysis of complex samples, 2D-LC can contribute to precision medicine approaches, such as identifying biomarkers for disease diagnosis, monitoring treatment response, and optimizing therapy. The integration of 2D-LC with portable devices and microfluidic systems can facilitate rapid and on-site analysis, bringing advanced analytical capabilities to the point of care.

COMPARISON OF 1D AND 2D LC

Aspect	1D LC	2D LC
Separation Power and Peak Capacity	Limited separation power and peak capacity	Enhanced separation power and peak capacity
Selectivity and Orthogonality	Limited selectivity based on stationary phase chemistry and mobile phase composition	Increased selectivity and orthogonality through different separation mechanisms
Compound Identification and Characterization	Limited capabilities for compound identification and characterization	Improved compound identification and characterization capabilities
Analysis of Complex Samples	Struggles with complex samples due to peak overlap and co-elution	Well-suited for complex sample analysis with reduced peak overlap
Method Development and Optimization	Optimization of mobile phase, column selection, and operating parameters	Optimization of multiple parameters for comprehensive analysis[22]

Applications of 2D-LC:

2D-LC has found widespread applications across various fields due to its enhanced separation capabilities and ability to analyze complex samples. Here is a review of some key application areas where 2D-LC has demonstrated its effectiveness:

1. Pharmaceutical Analysis:

2D-LC has proven to be valuable in pharmaceutical analysis, offering improved separation and detection of pharmaceutical compounds. It enables the analysis of complex drug formulations, impurity profiling, and the determination of drug-related substances. 2D-LC can also be utilized for the analysis of chiral compounds, enantiomeric purity determination, and stability studies. The comprehensive and orthogonal separations provided by 2D-LC contribute to more accurate quantification and reliable identification of target compounds in pharmaceutical samples.

2. Metabolomics:

Metabolomics aims to study the comprehensive profile of small molecules in biological systems. 2D-LC, with its high peak capacity and improved resolution, is well-suited for metabolomics applications. It allows for the separation and identification of a wide range of metabolites, facilitating metabolite profiling, biomarker discovery, and metabolic pathway elucidation. 2D-LC is particularly valuable when analyzing complex biological matrices, such as urine, plasma, or tissue samples, where numerous metabolites coexist.

3. Environmental Analysis:

2D-LC has demonstrated its utility in environmental analysis, particularly in the analysis of complex environmental samples. It enables the separation and identification of a wide range of contaminants, including persistent organic pollutants (POPs), emerging contaminants, pesticides, and metabolites. 2D-LC can provide improved selectivity and sensitivity for target analytes in environmental matrices, allowing for accurate quantification and characterization of pollutants.

4. Food and Beverage Analysis:

The analysis of complex food and beverage samples often requires high-resolution separation techniques. 2D-LC can effectively separate and characterize components such as food additives, natural products, flavors, and fragrances. It offers improved detection and identification of trace-level contaminants, enhances the understanding of food composition, and aids in quality control and authentication of food products.

5. Biopharmaceutical Analysis:

The analysis of biopharmaceuticals, including proteins, peptides, and monoclonal antibodies, requires advanced separation techniques. 2D-LC offers improved resolution and selectivity for the separation of these complex biomolecules. It enables the analysis of post-translational modifications, protein isoforms, and impurity profiling. 2D-LC coupled with mass spectrometry facilitates the characterization of biopharmaceuticals, including intact protein analysis and peptide mapping.[S

CONCLUSION

In this comprehensive review article, we explored the advancements, techniques, applications, and future perspectives of Two-Dimensional Liquid Chromatography (2D-LC). We discussed the various approaches in 2D-LC, such as heart-cutting, comprehensive, and online 2D-LC, and highlighted the importance of column technologies and stationary phases. Integration with hyphenated techniques and the potential of 2D-LC in personalized medicine and point-of-care diagnostics were also discussed. Method development strategies, applications in pharmaceutical analysis, metabolomics, and environmental analysis, as well as future trends, were examined. Overall, 2D-LC proves to be a powerful tool for complex sample analysis, offering enhanced separation, improved compound identification, and accurate quantification. It holds great promise for advancing scientific discoveries and addressing analytical challenges across various fields.

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