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Short communication

Artificial intelligence in HPLC method development: Opportunities, applications and challenges

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Introduction

High-performance liquid chromatography (HPLC) is one of the most versatile and widely applied analytical techniques in pharmaceutical analysis. Advances such as ultra-high-performance LC, mass spectrometry, and two-dimensional LC, significantly expand its analytical capabilities, but on the other side increase the complexity of method development, requiring simultaneous optimization of numerous interdependent parameters, especially in respect to complex matrices, or unstable analytes.

While computer-assisted approaches such as Design of Experiments and retention modeling have extensively been explored as tools for improving method development efficiency, recent advances in artificial intelligence (AI) have accelerated interest in AI-driven chromatographic optimization.

Applications of AI in HPLC method development

Among the most extensively investigated applications of AI in chromatography are the prediction of retention time and elution order. In comparison to conventional retention models, AI-based approaches have demonstrated superior ability to model nonlinear relationships between chromatographic variables and analytical outcomes. Machine learning (ML) approaches have been successfully applied to pharmaceutical small molecules (Abba et al., 2020), peptides (Žuvela et al., 2020) oligonucleotides (Enmark et al., 2022), and chiral compounds (Xu et al., 2023). The integration of molecular descriptors and stationary phase characteristics into ML predictive models has enabled substantial improvements in retention prediction accuracy and method optimization (Shi et al., 2025). AI has also been utilized for the prediction of optimal chromatographic conditions. ML-based platforms combining random forest classifiers and neural networks have been developed to predict suitable mobile phase compositions for reversed-phase HPLC analyses (Singh et al., 2025). These systems facilitate rapid identification of chromatographic conditions while reducing experimental burden.

AI methodologies have also been applied to chromatographic signal processing. Deep learning algorithms have demonstrated excellent performance in peak detection, peak integration, and baseline correction, particularly in complex or noisy datasets (Xu et al., 2021).

A further advancement is represented by closed-loop chromatographic systems integrated with ML algorithms, able to automatically explore chromatographic design spaces and identify optimal separation conditions with minimal human intervention, where Bayesian optimization strategies

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have shown particular promise in reducing the number of experimental runs, to achieve optimal performance (Bos et al., 2022, Dixon et al., 2024).

Challenges and limitations

Despite the significant potential of AI-driven chromatographic development, several challenges remain. The performance of ML models is strongly dependent on the quality and diversity of training data, with limited datasets reducing their ability to generalize across different compounds, laboratories, or chromatographic systems. Another important limitation is the lack of transparency associated with many AI models, particularly deep learning approaches. Their "black-box" nature presents challenges in highly regulated pharmaceutical environments, where method understanding and scientific justification are essential requirements. Inconsistencies in validation practices and limited availability of standardized benchmarking datasets hinder reproducibility and broader regulatory acceptance (Bhalerao et al., 2023, Nair, 2025). The computational demands associated with AI implementation may also limit accessibility, particularly for smaller laboratories lacking specialized infrastructure or expertise in data science and ML (Hanna et al., 2025).

Conclusion

AI-based approaches enable predictive modeling, automated optimization, and intelligent chromatographic data interpretation, demonstrating considerable potential for improved efficiency, and addressing complex chromatographic challenges that are difficult to resolve using conventional methodologies. Future developments are expected to focus on hybrid approaches that combine mechanistic chromatographic understanding with adaptive AI algorithms, providing a balanced framework for transparent and efficient chromatographic method development.

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