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Recent Updates in Chromatographic Techniques for Development of Drugs and Clinical Biomarkers

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Abstract

Copper nanoparticles (CuNPs) have attracted attention as versatile nanomaterials because of their unique physical, chemical and therapeutic properties quite different to those of bulk copper. Copper at the nanoscale possesses improved surface reactivity, redox activity and tunable optical and electrical properties that enable various technological and biomedical applications. This review includes a comprehensive summary of the physical aspects of CuNPs: size- and shape-dependent properties, crystallinity, optical response and thermal/electrical conductivities. Furthermore, the chemical properties of CuNPs including oxidation property, surface chemistry, catalytic activity and mechanisms associated with ROS generation and ion release were also reviewed in detail. Special consideration is given to the therapeutic role of CuNPs, including their anti-microbial/anti-viral activities, wound-healing properties, anti-cancer effects and drug-release applications. Tendencies and challenges for toxicology, biocompatibility and translation are also explored. In general, this review describes the most recent progress in CuNP research and provides future directions for the development of safe and effective therapeutic systems using CuNPs.

Keywords: Attitudes, Healthcare access barriers, Knowledge, Nigeria, Postnatal care, Practices, Urban-rural disparities

Introduction

Chromatographic methods are required for bioanalysis and have greatly advanced, and become most important in the emerging era of drug development and also clinical biomarker discovery. These techniques that split complex mixtures into their

components according to physico-chemical properties, are capable of accurate identification, quantification and characterization of analytes in drugs and substances having biological origin. With the introduction of hyphenated methods especially in line with mass spectrometry (MS), chromatography became increasingly important, changing analytical perception and response to challenges in pharmaceutical sciences, clinical diagnostics, translational medicine (Kaddah et al., 2025).

During drug development, chromatography methods are required at every stage of the research pipeline from early discovery and lead optimization studies to pharmacokinetics, metabolism studies and manufacturing quality control. Liquid chromatography (LC) with great popularity in drug bioanalysis, especially high-performance liquid chromatography (HPLC), and ultra-high-performance liquid chromatography (UHPLC) is proved to have superior separation performances and reproducibility of structurally varying drug candidates and metabolites from complex biological matrices (Tuzimski & Petruczynik, 2020). These strategies are applicable to key bioanalytical approaches quantifying drugs and metabolites with the required precision for an accurate establishment of preclinical, clinical ADME characteristics. In combination with tandem mass spectrometry (LC-MS/MS) these systems provide enhanced analytical performance by providing a high degree of selectivity and sensitivity for analytes even at levels too low to measure using conventional chromatographic or spectroscopic techniques (Mou et at., 2018).

The fusion of chromatography and mass spectrometry is one of the most important advances in analytical technology. Through the hybridisation of chromatographic physical separation with molecularly specific monitoring by MS, small molecule drugs and their metabolites, peptides and lipids can be analyzed robustly in complex biological systems. These hyphenated systems (e.g., LC–MS or GC-MS) allow multiple analytes to be detected and quantified concurrently in a single measurement, which is essential for the high-throughput drug screening, bioavailability studies, and metabolite profiling. pharmacyjournal. org This potential is especially useful in oncology and other therapeutic areas, particularly when fast, reliable determination of the drug concentration was used to optimize dose and assess safety (Patidar & Kamble, 2025).

In the field of clinical biomarker discovery, chromatographic techniques (particularly LC-MS/MS) have significantly changed the way biomarkers are identified and confirmed. Biomarkers are measurable aspects of biological processes (e.g., disease development) or response to treatment and many are present at low concentrations in fluids such as plasma, serum, urine, or cerebrospinal fluid. Chromatography−MS methods that can offer the requisite sensitivity and breadth of coverage are able to perform extensive profiling of the metabolome/proteome for disease-related chemical markers. The improved chromatographic resolution and the optimization of analytical parameters, in combination with novel data processing appliances, have improved metabolite coverage and facilitated identification of relevant diagnostic markers using LC-MS based metabolomics (Chen et al., 2023).

Furthermore, progressions in chromatographic separation methods (e.g., HILIC and LC-MS) have made it possible to separate the highly polar or structurally similar compounds that were not separable with the reverse-phase adequately previously developed for analyses on reversed-phase, such as multidimensional LC systems. These changes are especially important in the context of clinical proteomics and metabolomics, where subtle differences between molecular structure may be indicative for disease states or subtypes. Complementary approaches such as the use of sample derivatization and ion mobility spectrometry further enhance analytical precision and broaden the list of detectable potential biomarkers in clinical research (Chapel et al., 2023).

In addition to discovery, chromatographic methodologies may also be used for validation of potential biomarkers and clinical routine assays. The need for high specificity and throughput in clinical laboratories has promoted the use of LC-MS/MS as a primary platform for measurement of hormone, drugs-of-abuse, therapeutic drugs and endogenous biomarkers. These analyses require a sensitive chromatographic separation method with MS detection that is able to differentiate between target assay analytes and structurally similar compounds interference and matrix interferences from the biological matrix, so that accurate clinically relevant values can be achieved (Kaddah et al., 2025).

Further development of chromatography (e.g., UHPLC), integration of high-resolution MS, and automation of data handling also improved the performance analyzer to reduce runtimes and improve sensitivity as well as dynamic range. These developments can fulfill the ever-increasing demands on pharmaceutical R&D and precision medicine ecosystem, where rapid and precise measurement of medicines as well biomarkers are essential for drug dose determination leading to patient compliance (Patidar & Kamble, 2025).

These will not be the subject of this review, but by all these chromatographic methods and current detection systems (multi-mass technologies) drug discovery and clinical biomarker work today rest upon. With their high sensitivity, selectivity and rapid sample turnover rates these approaches are filling the information gap for drug characterization molecular profiling of disease signatures and personalized patient care. Ongoing advances in chromatographic science will hopefully provide further analytical capabilities to drive progress in pharmaceutical sciences and clinical diagnostics.

Gas Chromatographic Techniques in Drug Analysis

Gas chromatography (GC) is a widely popular analytical technique, which has a significant contribution in drug analysis

with special reference to volatile/semi-volatile or thermally stable compounds. Since introduction, GC has found widespread use in pharmaceutical sciences for qualitative and quantitative assay of drugs, impurities, residual solvents as well as metabolites. However, with the rapid development of liquid chromatography techniques and equipment, gas chromatography is still indispensable because of its high separation efficiency, sensitivity, reproducibility, and robustness as used in combination with selective detectors or/and mass spectrometry (Poole, 2018).

The basic principle of gas chromatography is the differential distribution of analytes between a gaseous mobile phase and liquid or solid stationary phase contained in a capillary column. The samples are vaporized in the injection port and then carried via an inert carrier gas (he is the most common, but nitrogen or even hydrogen will also work) through a column. Variations in volatility, polarity and interaction with the stationary phase provide different retention times enabling effective separation of compounds in complex pharmaceutical preparations. In this regard, GC is extremely well suited for the analysis of low-molecular-weight drugs, organic solvents, anesthetic agents, and steroids as well as degradation products generated during manufacture or storage (McMaster, 2019).

In pharmaceutical industry, GC is extensively used on the measurement of residual solvents as these are considered critical quality attributes by regulatory agencies such as ICH Q3C. Trace analysis of the organic solvents used in the synthesis or formulation is required to assess product safety. The gas chromatographic methodologies which include flame ionization detection (GC-FID) or headspace sampling (HS-GC) GC with FID are considered to be the reference for this purpose due to their high sensitivity and accuracy. Headspace GC, in particular, also provides great benefits due to lowered matrix interference and analysis of volatile solvents directly without the need of complicated sample preparation, which is very suitable for routine quality control laboratories (Kazakevich & Lobrutto, 2017).

Applications in pharmacokinetics and toxicology In the above, drugs and their metabolites in biological matrices e.g. blood, plasma urine or tissues are quantified by gas chromatography. Although derivatization to increase volatility and thermal stability is needed for most pharmaceutical compounds, improvements in both derivatization reagents and injection procedures have made the GC applicable to a wide range of drug molecules. The use of derivatization strategies (silylation, acylation or alkylation) enhances the sensitivity and chromatographic resolution of this approach for polar drugs with hydroxy, amino and carboxyl groups (Skoog et al., 2018).

Gas chromatography-mass spectrometry (GC-MS) is greatly strengthened its analytical power in drug analysis. Ight-Resolutio Chromatography GC and Chromatograph at the Massachusetts stitute of Technology as yet, a standard analytical method has been established for each type of abscis acid. GC-MS is commonly applied in the field of pharmaceutical research for identity verification of APIs, trace impurity identification, and degradation product characterization. The GC-MS is also reinforced as a confirmatory method by the existence of major mass spectral libraries, and makes it possible to compare with unknowns and correctly identify them (Thamer et al., 2023).

GC and GC-MS are commonly used in clinical and forensic laboratories for substances of abuse, anesthetics, and therapeutic drugs. On the other hand, their selectivity and sensitivity allow the detection of molecules at low concentrations, which is required for TDM and toxicological screening. Furthermore, the method has been widely applied in doping control and regulatory monitoring based on its sound validation history and recognition by international authorities (Poole, 2018).

In addition, new developments in the field of gas chromatography have contributed to improved analytical capabilities and throughput. High-resolution capillary columns, fast GC techniques and improved detector technologies have helped to reduce analysis time without compromising the quality of separation or even achieving better quality of separation (. In addition, combinations of automated sample introduction systems and sophisticated data processing programs have been developed to improve throughput and reproducibility in response to higher troughputs required for pharmaceutical analysis. Other eco-friendly aspects, for instance the utilization of hydrogen as carrier gas and low solvent volumes used elevated the green factor in current GC methodologies as well (McMaster, 2019).

Even in the face of liquid chromatographic competition, gas chromatography still maintains a key role in drug analysis for volatile analytes, residual solvent testing and confirmation. Its extensive regulatory approval, economics and compatibility with mass spectrometry also represent a testament to its ongoing relevance in pharmaceutical research and quality control.

Role of Chromatography in Clinical Biomarker Research

In clinical biomarkers research, chromatography as an essential analytical technology has been widely used for the discovery, validation and routine quantitation of biomarkers related to disease diagnosis, prognosis and treatment response. Clinical biomarkers, the quantifiable biological signs of normal or pathological responses to treatment or disease processes are frequently at low levels within complex biological samples such as plasma/serum, urine, saliva or cerebrospinal fluid. Bio-analytical quantitation of these biomarkers is crucial for understanding the metabolome changes in cancer patients.14 Chromatography techniques, which are capable of providing high selectivity, sensitivity and reproducibility are essential to accurately measure/determine these bio-markers and have

become an indispensible tool in modern clinical research and translational studies (Tuzimski & Petruczynik, 2020).

In chromatographic approaches, liquid chromatography (LC), especially high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography (UHPLC), is now recognized as the leading platform in clinical studies on biomarkers. These methods facilitate efficient separation of a variety of biomolecules such as metabolites, lipids, peptides and low-molecular mass proteins under mild conditions suitable for biological samples. Liquid chromatography interfaced with mass spectrometry (LC-MS or LC-MS/MS) facilitates the simultaneous separation of physical and molecular properties, enabling mass analyzers to measure both the structure and presence of a molecule. This technological merger has significantly developed the field of biomarker analysis to allow targeted, semi-targeted and untargeted quantitation approaches all in a single method (Theodoridis et al., 2012).

In biomedical science, chromatography plays an important role in metabolomics and proteomics to deine molecular signature associated with disease states. LC-MS-based metabolomics coupled with an optimized chromatographic separation allowed systematic analysis of endogenous metabolome derived from diverse chemical classes. Developments in stationary phase chemistry, gradient elution procedure and multidimensional chromatography now allow the possibility of covering a larger number of metabolites that will increase the ability to detect more subtle biochemical changes associated with cancer, cardiovascular disease metabolic disorders and neurological diseases. Chromatographic separation is also crucial for proteomics workflow since the peptide mix that has been digested enzymatically must be very well separated at high resolution before MS analysis so as to obtain accurate protein IDs and quantification (Chen et al., 2023).

Biomarker validation is playing as another gate between discovery type work and clinical use, with chromatography very involved in this also. Validation requires a very measurable, precise and reproducible quantification of the putative (potential) biomarkers in big sets of samples. LC-MS/MS methods are emerging as the reference standard in this area of measurement due to their superior analytical specificity, which allows for reduced cross-reactivity (as is common with immunoassays). By means of the providing chromatographic separation, matrix interference is effectively removed and further ensures that biomarkers like steroidal hormones, bile acids, amino acids and therapeutic drugs can be quantified accurately in clinical samples. This stability is essential to demonstrate the strength of biomarker and clinical utility (Grebe & Singh, 2011).

Besides the LC-based strategies, gas chromatography (GC) is invaluable for some classes of clinical biomarkers, like volatile and semi-volatile compounds. GC-MS has particularly dominated in clinical metabolomics research for organic acids, fatty acids and small volatile metabolites of relevance to metabolic disorders and inborn errors of metabolism. The excellent resolution and extensive spectral library of GC-MS ensures confident identification of compounds, making it an important headspace sampling approach for research investigations as well as clinical laboratory analysis. Although derivatization is usually required, advances in sample preparation have increased throughput and repeatability, maintaining GC as a contemporary approach for marker discovery (Dunn et al., 2011).

There are also other emerging chromatographic applications in therapeutic monitoring and personalised medicine. Achieving this equilibrium is even more critical for treatment monitoring in medicated diseases, since metabolic status of patient with a specific disease under medication conditions dynamically and bio-analyte quantification (i.e.,drug metabolites, endogenous homeones or disease-specific metabolic) used to monitor drug effectiveness should be accurate to ensure correct therapy and minimise side effects. Chromatographic methods, notably LC-MS/MS, have become widely established in the majority of clinical laboratories for routine measurements of biomarkers for various purposes (endocrinology, oncology, and cardiovascular disease). Further, due to chromatography's ability to identify more than one established biomarker in one assay this paves the way for multiplexed panel analysis which is important not only for patient stratification but also precision medicine programmes (Grebe & Singh, 2011).

The lately developed technology increasingly contributes to the importance of chromatography in clinical biomarker analyses. Innovations such as UHPLC, HRMS, IMS and automated sample preparation systems have greatly improved the sensitivity, throughput and reproducibility of analysis (. In addition, the recent growth in bioinformatics and data structures has enabled us to understand complex chromatography datasets more completely and also with greater confidence to identify and validate biomarkers. Such advancements are very pertinent for clinical research, with its emphasis on large cohorts and longitudinal designs (Gika et al., 2019).

There are however several issues that need to be solved including method harmonization, inter-laboratory reproducibility and a regulatory approval for biomarker tests based on chromatography. However, continuous optimization of analytical processes such as SM and RM also serve to enhance the clinical applicability of chromatography (Tuzimski & Petruczynik, 2020).

Future Technical Innovations in Chromatographic Bioanalysis

Responding to the increasing sophistication of pharmaceutical research, clinical diagnosis and precision medicine, chromatographic bioanalysis has been dynamically developing. As an increasing number of complex biological sample types are being analyzed, and as the analytical questions faced become more challenging, future advances in chromatography will be driven by developments offering improved levels of sensitivity, selectivity and throughput while addressing issues associated with robustness (e.g., miniaturization), process efficiency (reduced sample volume/analysis time) and green aspects (environmental impact). Recent developments in instrument technology and microflow systems, stationary phases, hyphenated techniques, automation and data treatment are changing the face of chromatographic bioanalysis and enlarging its role in drug discovery and clinical biomarker research (Gika et al., 2019).

The advancement of ultra-high-performance liquid chromatography (UHPLC) is a forefront trend of future development of chromatographic bioanalysis. UHPLC Pressure Over the years, high-pressure UHPLC systems with sub-2 μm and superficially porous particles enable greater separation efficiency and faster analysis compared to HPLC. Recent advances have attempted to stabilize the column, reduce extracolumn dispersion and permit ultrafast gradients that elicit retention similar to <10 seconds. For high throughput bioanalysis where fast and reproducible determination of drugs or biomarkers in large clinical studies is needed (Nováková & Vlčková, 2009).

Another important development is multidimensional chromatography, for example two-dimensional liquid chromatography (2D-LC). This approach employs two orthogonal separation mechanisms of reverse selectivity, and, therefore, has higher theoretical peak capacity leading to more comprehensive profiling for complicated biological mixtures. Not only, 2D-LC is getting an increasing attention in bioanalysis for proteomics, metabolomics and impurity profiling due to the inability of single LC dimension to meet their analytical needs. Work is continuing on newly-engineered versions of the method for ease of application and robustness, adaption to mass spectrometry, so that it can be used as a routine clinical diagnostic tool, and for the testing of pharmaceutical compounds (Stoll et al., 2007).

The coupling of chromatography to high resolution mass spectrometry (HRMS) is a further, revolutionizing development. HRMS offers exact mass measurement and structural information alongside chromatographic separation, which allows for an unambiguous assignment of unknown compounds as well as biomarkers. Enhancements in orbitrap and time-of-flight (TOF) technologies as well as chromatographic performance sustain non-targeted and semi-targeted bioanalysis. Sensitivity, matrix effects, and quantitative robustness in clinical biomarker analysis (Grebe & Singh, 2011).

20 Miniaturization and microscale chromatography are also becoming significant trends. Micro-LC, nano-LC, and chip chromatography are techniques that decrease the amount of solvent and sample needed for analysis as well as improve sensitivity, especially when integrated with MS detection. They are particularly appropriate for applications where sample is precious, such as with paediatric studies, tissue biopsies and single-cell analysis. Further advances related to microfluidic design and column manufacturing are anticipated to increase the ruggedness and extend the clinical utility of miniature LPLCs (Karger & Snyder, 2018).

Automation and 'smart' sample preparation are continuing to dominate the future of chromatographic bioanalysis. Sample processing continues to be one of the largest sources of variation and error in bioanalysis system working parameters. The design of fully automated systems with SPE, online sample cleanup and direct injection systems is a goal to improve the reproducibility and throughput. By integrating the automated PP directly with chromatographic systems, human work is minimized, the turnaround time is shortened and standard clinical assays are facilitated (Tao et al., 2011).

Concurrently, greener and more sustainable chromatography is becoming increasingly significant. New developments focus on low solvent consumption, eco-friendly mobile phases and alternative carrier gases. The substitution of ethanol as a mobile phase, supercritical fluid chromatography (SFC) and energy-saving equipment places chromatographic bioanalysis in the context of global sustainable objectives without prejudice to its analytical capability. SFC in particular appears to be a promising choice for bioanalysis, on account of its high separation speed, low solvent consumption and compatibility with MS detection (Perrenoud et al., 2014).

One of the most disruptive advances in the near future might be related to data processing and AI incorporation. Current chromatographic bioanalyses produce massive complicated data that can't be interpreted without the use of advanced computational equipment. ML and AI-based algorithms are more and more involved in chromatographic peak identification, alignment, deconvolution and quantification. They facilitate reduction of poor data quality and analyst bias, as well as aiding in repositioning research towards being amenable for real-time decisions within high-throughput settings. Applications of AI - enabled analysis in biomarker discovery In the search for bio markers, AI driven an alysis improves pattern recognition and provides assistance to reveal clinically meaningful molecular signatures from primary chromatograms at scale (Gika et al., 2011).

Conclusion

Gas chromatography is still indispensable for drug analysis: for Drug discovery, pharmacological routine and on patient samples. New technical upgrades and methodical

developments will continue to increase the applicative capabilities of GC and GC-MS in today's pharmaceuticals and biomedical sciences. The discovery and validation of biomarkers based on clinical studies, as well as their routine use in the clinic is heavily dependent on chromatography. High resolution separating power, accurate quantification and compatibility with state-of-the-art detection techniques are the characteristics that make CE non-isotachophoresis a suitable analytical tool to meet modern diagnostics and personalized medicine demands. As chromatographic science continues to advance, this will increasingly drive the current stable of biomarker research methodologies and affect disease diagnosis, prognosis, and treatment. Horizon of future technical advances in chromatographic bioanalysis Increasing the analysis speed, the sensitivity and coverage collectively power driving forces for technical evolution of chromatography for bioanalysis. Advancement in UHPLC, multidimensional separations, HRMS interfacing, operation scaling-down and palliant automation to provide “green” chromatography environment; implementation of AI-supported data processing. These developments are expected to significantly enhance drug discovery, clinical biomarker discovery and precision medicine indicating the continued critical role of chromatography in life science research.

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