

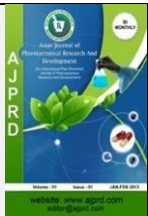


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Review Article

Gap Analysis and Recent Advances in HPLC and UPLC

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ABSTRACT

Objective: To systematically review the advances of High-Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography (UPLC) since the year of 2020 in the fields of analytical performance, sustainability, automation, and emerging technologies.

Data Sources: Databases were searched for all literature related to HPLC and UPLC published from 2020 to 2026 using Scopus, PubMed, Web of Science, and Google Scholar as resources.

Study Selection: A total of fifty-five peer-reviewed papers were chosen based upon predefined eligibility criteria to be included as part of this literature survey these comprised fifteen reviews, twenty-nine articles, and eleven abstracts.

Summary of Contents: Advances in the following areas are reviewed: Sub-2 μ m and superficially porous columns, Q-TOF MS, green analytical chemistry metrics, automated processes, AI, and miniaturized sample preparation. Challenges include limited transfers of existing methods, lack of open access databases, and inadequate validation of AI-assisted method development by regulatory bodies.

Conclusion: HPLC and UPLC have the potential to evolve into faster, more eco-friendly, and innovative analytical platforms. Addressing current limitations will allow for further integration of HPLC and UPLC with nanotechnology, microfluidics, and sustainable analytical chemistry.

Keywords: High-Performance Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC), Green analytical chemistry, Machine Learning

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INTRODUCTION:

Fundamentals of HPLC and UPLC:

In the late 1960s, the modern form of liquid chromatography was introduced, as a result of research carried out by Horvath, Kirkland and Knox, who discovered that reducing the diameter of the stationary phase dramatically increased the efficiency of the column. The Van Deemter equation, modified to fit fluid phase systems, predicts that, as the diameter of the particles decreases, the height equivalent to a theoretical plate (HETP) will also decrease, provided that there is sufficient pressure to maintain a high enough velocity of the mobile phase through the column. (1) Traditional HPLC, which uses 3-5 μ m particles at a maximum pressure of 400 bars, has been able to provide resolutions and throughputs that meet requirements from the pharmaceutical and environmental regulatory authorities for many years. In

2004, the Waters Corporation commercialised the use of sub-2- μ m particles, branded as "UPLC", and other companies, including Agilent (using the term UHPLC), Shimadzu (using the term UFLC) and Thermo Fisher Scientific (using the term UHPLC), have subsequently added their own product offerings based on sub-2- μ m particle technologies; these developments have dramatically changed the expectations for analysis time, solvent usage and peak capacity. (2)

UPLC utilises the advantages of low plate height inherent in using sub-2- μ m particle size particles by running at 600–1,500 bar pressure and requiring specific instrument platforms that employ either a high-pressure binary or quaternary pump, low dispersion fluidic circuitry, and a fast scanning detector. (3) As a result of these changes, the run times for UPLC versus traditional HPLC are reduced by a factor of 3 to 10, and there is a corresponding improvement in mobile phase consumption (e.g., 0.1 μ L for

HPLC vs. 0.5 μ L for UPLC), as well as significant improvements in peak height sensitivity (i.e., narrower peak widths). Thus, these characteristics have been instrumental in increasing UPLC use in pharmaceutical QC laboratories, bioanalytical research laboratories and environmental monitoring agencies that are both limited by sample throughput as well as increasing sensitivity of analysis. (4)

Importance Across Analytical Domains:

The pharmaceutical industry is reliant upon HPLC and UPLC methods to meet regulatory requirements defined by the ICH (International Conference for Harmonisation). HPLC and UPLC analyse the Assay of an Active Pharmaceutical Ingredient (API); conduct Impurity profiling; prepare for dissolution testing; prepare for cleaning validation; and analyse pharmacokinetic bioassay/clinical studies for regulatory agencies requiring compliance with the ICH guidelines (i.e. Q1, Q2(R2), and Q3). The performance expectations of regulatory agencies require that we demonstrate Method suitability using the System Suitability Testing (SST) parameters, i.e. Theoretical Plate Count, Tailing Factor, Resolution, and Relative Standard Deviation. The performance of the instrument has a direct correlation to regulatory compliance. The ICH 2023 revised guidelines (Q2) recognise UPLC as an accepted analytical methodology. This recognition is a major factor for the increased usage of UPLC in new drug applications. (5-7)

HPLC and UPLC are critical tools in environmental analytical chemistry for measuring pesticide residues and other contaminants, including pharmaceuticals and PFAS, in various environmental matrices such as water, soil, and living organisms. These techniques are essential to meet detection limits below one part per trillion (ppt), as mandated by European Union Directive 2020/2184 for drinking water quality, and by the US EPA for its 2023 PFAS National Primary Drinking Water Regulation. HPLC and UPLC are equally valuable tools for food safety, where multi-residue methods that identify hundreds of analytes in one run are now the industry standard. UPLC has significantly improved throughput over traditional HPLC methods by utilising UPLC-Q-TOF, which allows for non-targeted analysis with the same level of sensitivity as traditional targets. (8,9)

The bioanalytical applications of UPLC continue to evolve as the technology has allowed for rapid resolution of structurally similar compounds that are found in biological samples (<3 minutes). The marriage of UPLC with recent advances in high-resolution mass spectrometry (HRMS) instrumentation (Orbitrap and Q-TOF) has allowed for the completion of untargeted metabolic phenotyping studies, which would typically take an overnight run on HPLC, in as little as one hour. (10)

Rationale for a Gap Analysis:

Even if the number of published articles about methods has increased significantly, there remain limited structured gap analyses of what HPLC and UPLC methods can consistently achieve and where there is no consensus in the community about the techniques (i.e., no agreement on how well each of these methods works). This is in part because

the literature that exists in this area presents mainly narrative summaries of method improvements or advances without offering critical evaluations of unaddressed issues. Additionally, with the expectations placed upon manufacturers regarding lifecycle management by regulators under ICH Q14, and the expectations placed upon manufacturers by regulatory agencies regarding harmonised multi-laboratory validation data, an accurate assessment of the limitations of these techniques will have important implications for both academic and industry researchers. With that in mind, the purpose of this article is to assess not only the improvements in the testing methodology but also the quality of the methodologies reported in the literature; to highlight the inconsistencies between published studies using the same technique(s); and to develop a ranked research agenda for the next 10 years. (11-14)

Methodology of Review

Search Strategy

We performed a systematic review of literature from January 2020 through April 2026 by searching four electronic databases: Scopus (Elsevier), PubMed (National Library of Medicine), Web of Science (Clarivate) and Google Scholar. The search strategy involved employing the Boolean operators with some combination of the following primary terms: ("HPLC", "high-performance liquid chromatography", "UPLC", "ultra-performance liquid chromatography", and "UHPLC") AND ("method development", "column technology", "green chromatography", "pharmaceutical analysis", "environmental analysis", "food analysis", "bioanalysis", "mass spectrometry", "artificial intelligence", and "machine learning"). Secondary searches then targeted specific subtopics, including: "superficially porous particles", "core-shell columns", "sub-2-micron", "AGREE metric", and "UPLC-MS/MS validation". (15,16)

Inclusion and Exclusion Criteria:

Any research published between 2020 and 2026, written in English, published in a peer-reviewed journal, and presenting an original experimental design or collection of data, was used as part of this review. To qualify, research must have had to be a) available in complete form using either free dissemination on the internet (e.g., open access), or through an institutional subscription; b) written within 18 months preceding the date of this review; c) included original research, or systematic reviews, as well as narrative reviews based on authoritative sources; d) relating directly to either HPLC & UPLC instrumentation and/or method development using HPLC & UPLC; and e) cited within an appropriate reputable source such as Pub Med/Pubmed Central, Scopus, Web of Science, or the National Library of Medicine. (17,18)

Any record that was related to any one of the following four areas was not eligible for inclusion: a) use of ion chromatography, gas chromatography, or supercritical fluid chromatography exclusively as compared with HPLC or UPLC; b) presentation of conference abstracts, letter(s), editorials and patent documents that either contain no references to peer-reviewed literature, or do not contain adequate evidence to substantiate the usefulness or

reliability of any method or result as validated against a standard of the International Conference on Harmonisation (ICH) Q2(R2) Guidance Document for the Validation of Analytical Method(s), or equivalent regulatory authority or c) duplicate records being identified across different databases. (19-21)

Study Selection and Quality Assessment

A total of 1,847 unique records after de-duplication were reviewed against pre-set inclusion/exclusion criteria for titles and abstracts independently. This resulted in the selection of 312 articles for full-text review. Ultimately, 55 studies totally fulfilled the inclusion/exclusion criteria. All of these were the evidential foundation for this review. (22) The quality of quantitative method articles was evaluated using an adapted version of the AGREE II framework (specifically focused on validation-related items, including linearity, accuracy, precision, limit of detection, limit of quantitation, specificity, and robustness) while review articles were assessed based upon the AMSTAR-2 standards. (23)

Fundamentals and Comparative Analysis of HPLC and UPLC

Chromatographic Theory

The efficiency of a column used for chromatography can be represented as theoretical plates (N). Theoretical plates (N) are calculated as peak width at half-maximum divided by retention time. The Van Deemter equation ($H = A + B/u + C_u$) describes how eddy diffusion (A), longitudinal diffusion (B/u), and mass transfer resistance (C_u)

contribute to broadening of bands as a function of the linear mobile-phase velocity (u). Of most importance in considering rapid separations is the C-term, since it is the largest contributor to band broadening under the high velocities needed for rapid separations, and it decreases as a function of dp^2 when dp is halved. For example, by going from a particle diameter of 5 μm to 2.5 μm , one sees a four-fold reduction in C, which helps to explain how UPLC columns with particles less than 2 μm in diameter are still efficient at twice to three times the flow rates of conventional HPLC columns. (24,25)

Peak capacity (P_c) or the number of distinct peaks that can be distinguished from one another in a retention time window is related to both the gradient elapsed time and column efficiency. The ringed UPLC systems can produce more numbers of N/second leading to peak capacities between 400-600 peaks for a 10-minute gradient, whereas the equivalent HPLC systems yield peak capacities between 150-300 peaks for the same 10-minute gradient. (26) This outstanding difference represents a significant, if not critical, advantage for metabolomic and proteomic workflows, given the myriad of potential analytes that need to be characterised. The kinetic performance limit (KPL) framework developed by Desmet and coworkers provides an elegant, pressure-normalised measure of efficiency that enables one to objectively compare multiple instruments across platforms and validates that sub-2 micron fully porous media and 2.7 micron superficially porous (core-shell) media are equivalent regarding kinetic performance under the available pressures of the current UPLC instrument. (27,28).

Instrumentation Differences

Table 1: Comparative instrumentation specifications: conventional HPLC vs UPLC/UHPLC

Parameter	Conventional HPLC	UPLC / UHPLC
Particle diameter	3-5 μm	< 2 μm (FPP); 2.6–2.7 μm (SPP)
Operating pressure	Up to 400 bar	600-1500 bar
Typical flow rate	0.5-2.0 mL/min	0.3-0.6mL/min (2.1 mm ID)
Column internal diameter	4.6 mm (standard)	2.1 mm (standard)
Theoretical plates	50,000-100,000	150,000-300,000
Typical run time	15-60 min	2-10 min
Solvent Consumption	High (10-50 mL/run)	Low (0.5-3 mL/run) Narrow peaks, higher sensitivity
Detector response	Broader peaks, lower height	Narrow peaks, higher height sensitivity
System dwell volume	> 1000 μL	< 200 μL
Capital cost	Moderate	High (1.5-3 $\times$ HPLC)
Maintenance complexity	Lower	Higher (seals) (29-32)

In addition to using smaller particles, UPLC uses different mechanical designs than HPLC. The volume of fluid between the point of mixing the gradient mobile phase and the inlet of the column is known as "dwell volume." To prevent gradients from becoming distorted when using very low flow rates, the dwell volume of UPLC systems needs to be as small as possible. The dwell volume of UPLC is less than or equal to 200 μL , whereas the dwell volume of

regular quaternary pump HPLC systems is generally between 600 and 1,500 μL . UPLC uses pulse-damping technology to reduce high-frequency fluctuations in pressure generated by the reciprocating motion of the piston; baseline noise introduced by these fluctuations is more pronounced with narrow peaks than wider peaks. The cells where the detectors are located also need to be small; for example, the photodiode array (PDA) detector cells are

typically in the 500 nL range, and the fluorescence cells are approximately 1 mL, as opposed to the standard size of 8-13 mL for the same detectors in a traditional HPLC scheme. (33-36)

Efficiency, Resolution, and Selectivity

The fundamental resolution equation, which describes resolution (R_s) between two contiguous peaks, is a combination of three variables—selectivity (α), retention factor (k), and efficiency (N)—that work together to define resolution. The use of UPLC is advantageous in terms of efficiency through its N -term, and consequently will improve R_s directly. It is important, however, to note that selectivity—the way a stationary phase interacts with a mobile phase—determines the separation between peaks that have similar k values as a result of differing efficiencies in both HPLC and UPLC systems. (37) A very important piece of information that has arisen from research published between 2020 and 2024 concerns the fact that, although many manufacturers produce C18 stationary phases, differences in selectivity between these products will sometimes exceed the degree of selectivity difference that would result from different particle sizes. Therefore, transferring HPLC methods to a UPLC system solely based on a comparison of efficiencies can be very misleading. (38)

Recent Advances (2020-2026)

Column Technology Innovations

Superficially Porous Particles

Superficial porous particles (SPPs), also called core/shell or fused core particles, are made up of a solid silica core enclosed by a thin porous shell about 0.2-0.5 μm thick. This design permits SPPs to achieve the same efficiency as fully porous sub-2- μm particles, and with much lower backpressure. (39) From 2020 to 2024, SPP technologies expanded from almost exclusively C18 phases to include polar embedded, phenyl-hexyl, biphenyl, pentafluorophenyl (PFP), and mixed mode particles, providing additional selectivity options for analysing complex sample types. An example of this progress was demonstrated by Cunha et al., who found that 2.7- μm SPP columns produced peak capacities equivalent to those achieved with 1.7- μm fully porous columns when analysing polyphenols from plant extracts and operating at an overall system pressure that was 40% less than that utilised with 1.7- μm fully porous columns. (40) This finding will have a direct impact on laboratories using conventional HPLC instrumentation. Hernandez-Mesa et al. validated an SPP-based UPLC-MS/MS method for analysing multi-class mycotoxins in cereals using a limit of quantitation of 0.1-2.5 $\mu\text{g/kg}$ and run times of less than 8 min., performances that could not be achieved using 5- μm HPLC columns. (41)

Novel Stationary- Phase Chemistries

The hybrid technology charged surface hybrid (CSH) with positively charged sites on the surface, preventing silanol activity at low pH (or acid) conditions, is now the preferred stationary phase for analysis of basic drugs in UPLC, allowing for peak asymmetries less than 1.2 (asymmetry factor) for the same pH: the range of pH is 2-8 without the

need for ion-pairing reagents. Hybrid organic-inorganic particles consisting of bridged ethylene silica (BEH) in the matrix have been created to improve column stability at extreme pH and temperature conditions; BEH columns can have a lifetime greater than 10,000 injections under the most extreme mobile phase conditions. Zwitterionic (ZW) HILIC Columns achieved new levels of aqueous-phase retention of polar species, including nucleotides, amino acids, and glycols, between 2022 and 2025; this represents the first time that polar compounds can be retained in reverse-phase chromatography. (42-44)

Monolithic and 3D- Printed Columns

Silica monolithic columns have experienced renewed attention (2020–2024) from improvements made to reproducibility of silica condensation protocols because they are bimodal (macroporous& mesoporous), allowing the use of higher flow rates & lower back pressure. (45) Carbonell et al. developed monolithic columns with 10 μm domain size, attaining theoretical plate count of 3 μm particle packed columns, while utilizing one-fourth the back pressure. The introduction of additive manufacturing (3D printing) for the fabrication of columns signifies a paradigm shift; printed polymer columns with photopolymerised monolithic phases demonstrated chromatographic resolution for pharmaceutical impurities at proof of concept by 2023, although there is still considerable batch-to-batch variability in porosity that limits acceptance. (46,47)

Detector Advancements

Mass Spectrometry Detectors

Coupling ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry (MS/MS) in a triple quadrupole (QqQ) configuration has become the default method for quantitative bioanalysis and residue analyses. The addition of atmospheric pressure chemical ionisation (APCI) to electrospray ionisation (ESI) has expanded the range of analytes amenable to analysis in a single chromatographic run to include fat-soluble vitamins and triterpenes, which are non-polar compounds. The enhancements in sensitivity of triple quadrupole systems from 2020-2026 resulted in the attainment of lower limits of quantification (LLOQs) of 0.001 to 0.01 ng/mL for high-priority metabolite drugs in human plasma, thereby meeting the EMA's and FDA's bioanalytical guidelines for LLOQ; previously, these had to be pre-enriched by an immunoassay before being run on a triple-quadrupole system. (48-50)

Mass spectrometry coupled with ultra-performance liquid chromatography (UPLC) has enabled non-targeted analyses to be accomplished at a high level of precision. Orbitrap instruments provide a resolving power of 240,000 FWHM at m/z 200 and are thus able to confidently assign elemental composition of unknowns under 500 Da, and have been used extensively for identification of pharmaceutical degradation products produced from exposure to photostability stresses as defined by ICH Q1B. Q-TOF instruments have relatively quick data acquisition rates compared to other systems (100 spectra/second), and possess resolving powers of the 30,000-50,000 range, making them highly desirable in the metabolomics

workflow due to their ability to rapidly switch between positive and negative ion modes. (51) Since 2023, trapped ion mobility spectrometry (TIMS) in combination with Q-TOF (timsTOF) has introduced another collisional cross-section dimension into UPLC-MS analysis, allowing for further separation of isobaric species, which would otherwise co-elute chromatographically. (52)

Photodiode Array and Fluorescence Detectors

Even though mass detection methods are becoming more common, PDA detectors are still widely used by quality control laboratories when checking for APIs that absorb UV light, as they provide inherent spectral confirmation without the complications of maintaining an immediate source of ionisation. (53) The additional enhancements made to fibre-optic PDA cells from 2020 to 2024 increased optical path length up to 10mm using Z-cell configurations, increasing sensitivity approximately 10 times compared with a traditional 1mm cell. With regard to substances that naturally fluoresce/are labelled with a fluorescence marker, fluorescence detection remains superior with respect to sensitivity; recent developments in LED based fluorescence detectors (2021 to 2024) have resulted in the ability to reach LOD of 0.01–0.1 fg on-column for fluorescein tagged antibody fragments - this new technology has outperformed traditional lamp based systems and has greatly reduced both energy consumption and maintenance cycle times. (54,55)

Charged Aerosol and Evaporative Light Scattering Detection

The need for a universal detection method for analytes that do not absorb ultraviolet light (UV), like excipients, sugars, and lipids, is long-standing. As such, charged aerosol detection (CAD) and evaporative light scattering detection provide two different ways to detect such compounds. CAD has replaced ELSD in most pharmaceutical applications due to its greater sensitivity, ranging from 10 to 100 times greater than that of ELSD, along with a broader dynamic range and more consistent response behaviour. The Vanquish CAD detector from Thermo Fisher (released in 2021) demonstrates a linear range of 3 orders of magnitude for polysorbate 80 contained in biopharmaceutical formulations — a molecule that does not contain an absorbing chromophore — allowing it to be quantified without the need for derivatisation. (56,57)

Green Chromatography Approaches

Green analytical chemistry is concerned with identifying ways to minimize the consumption of hazardous solvents, reagent toxicity, energy use, and waste generation while still providing similar performance characteristics for the analysis. In the United States, four structured methodologies evaluate the environmental impact of HPLC and UPLC methods: the National Environmental Methods Index (NEMI), the Analytical Eco-Scale, the GAPI (Green Analytical Procedure Index), and the AGREE metric, all of which provide a structured scoring system. (58) Between 2020 and 2025, the AGREE metric has been validated against 120 published pharmaceutical analytical methods and has been determined to be able to discriminate between true green analytical methods and those that have been superficially modified; therefore, the implications for the

requirements for submission of manuscripts to journals are evident. (59)

From 2021 to 2025, ethanol (EtOH), isopropanol (IPA), and acetonitrile were assessed for use as mobile-phase components in UPLC separations for bio-based substances. Isopropanol provided equivalent resolution to acetonitrile at 35°C when separating lipophilic pharmaceutical compounds and resulted in 25% less global warming potential over 12 months (assessed using life cycle analysis methodology. This also allows for new aqueous-ethanol mobile phases to be confirmed for the reversal separation of polyphenolic antioxidants with non-toxic AGREE scores, on average 30% greater than an acetonitrile-based mobile phase. Both the high viscosity of ethanol and the adaptability of seal and rotor material will need to be verified before use — a large number of published procedures do not provide sufficient evidence for this verification. (60-61)

Automation and Artificial Intelligence Integration

In the early 2010s, automated scientific method development tools that use design of experiments, multivariable optimisations, have been on the market; and now that they are integrated with machine learning algorithms, they represent a major improvement in use and application in today's science and technology (2020s). DryLab 4 (Molnár-Institute), ChromSword (Merck) and many other open-source Python-based scientific optimisation platforms can use gradient modelling and integration of Bayesian methodologies with the modelling, thus providing the capability to estimate optimum gradient profiles (U. P. 7) based on less than 10 scouting experiments. A Gradient Boosted Decision Tree (GBDT) model was used to predict UPLC retention time (abs. error < 0.15 min) based on a total of 2400 retention times from structurally diverse pharmaceutical products. As a result, there was a reduction in the number of scouting experiments by 8 or 4. (62-64)

Beyond just method development, there are many other applications of AI as well, including, but not limited to, spectral interpretation, peak integration, monitoring system suitability, and predicting the rate of failure. Using CNNs to analyse UPLC chromatographic fingerprints, it has been possible to automatically categorise 47 different botanical extract species with a level of accuracy of 96.8%, where previously this would have required expert judgment to manually identify patterns. In pharmaceutical manufacturing, machine-learning models have been developed to predict column failure (which can be done based on 14 different UPLC-system parameters: pressure, temp., gradient delay volume, and detector noise) an average of 4.2 days ahead of when peak-broadening could be detected via the application of conventional SST criteria; such results have significant implications for ensuring quality of the continuous manufacturing process. (65,66)

Miniaturisation and High- Throughput Techniques

With the advancement of Nano-HPLC & capillary UPLC (using 75–300 µm internal diameter columns at 200–400 nL/min flow rates), these technologies have become critical components of proteomic workflows, especially when

dealing with sample amounts limited to femtomole quantities. (67) Chip-based liquid chromatography, which combines the functions of columns, injectors, & splitters onto a single microfluidics chip, has been granted regulatory clearance from the FDA 510(k) for bioanalytical purposes following clinical mass spectrometry regulatory clearances from 2021 through 2023. (68) A parallel

column approach involves running multiple (4 –8) independent UPLC columns concurrently on a single instrument. Agilent has developed this technology (InfinityLab® Poroshell) to deliver sample throughput rates of 96 samples/hour for API purity testing, equating to 4 hours of continuous use of a conventional UPLC instrument. (69).

Applications: Selected Highlights

Table 2. Selected HPLC/ UPLC method applications

Sector	Analytes	Platform / Column	Key Achievement	Citation
Pharma QC	14 API impurities (ICH Q3B)	UPLC BEH C18, 1.7 µm	< 6 min; LOQ 0.005%	70
Bioanalysis	Antivirals in plasma (COVID-19)	UPLC-MS/MS CSH C18	LLOQ 0.5 ng/mL, 4 min	71
Environment	PFAS (24 congeners) in water	UPLC-Q-TOF, PFP column	LOQ 0.4 ng/L	72
Food safety	Mycotoxins (multi-class)	UPLC-MS/MS SPP C18	LOQ 0.1–2.5 µg/kg	73
Metabolomics	Plasma metabolome (>800 features)	UPLC-Q-TOF, HILIC + C18	Pc > 450, 12 min	74
Forensics	40NPS (novel psychoactive)	HPLC-PDA-MS, PFP	98.4% correct ID	75
Biopharm	mAbcharge variants	HPLC IEX, pH gradient	Resolution > 2.0, 20 min	76
Clinical	25-OH-Vitamin D2/D3 in serum	UPLC-MS/MS, PTAD deriv.	LOQ 1.25 nmol/L	77

Pharmaceuticals

There has been a significantly increased use of UPLC instead of HPLC in the pharmaceutical industry across the board over the time frame between 2020–2026. Orlandini and colleagues performed a series of comparative experiments using 200 different commercial tablets to see how they could reduce analyte time with both HPLC and UPLC methods at the same time, with an analysis time reduction of 68 percent (median) and maintained at least equal to or more accurate than stated certified (mean bias < 0.5 percent versus reference values certified). For biopharmaceutical characterisation, both charge variant determinations, size exclusion determination and glycan profiling of monoclonal antibodies depend mostly on the use of HPLC ion-exchange and HILIC type platforms in a non-denaturing state, which may show incompatibility due to pressure constraints imposed by UPLC method requirements, damaging the conformation of the large molecules that are part of the process. (78,79)

Environmental and Food Analysis

During the 2020–2026 period, the application of ultra-performance liquid chromatography (UPLC) for the analysis of emerging environmental pollutants has increased substantially. Spataro et al. developed a high-throughput method for the analysis of 24 PFAS compounds in potable water utilizing an UPLC-MS/MS. The authors report that matrix-matched calibration was conducted, recovery values were between 89 and 112 %, and limits of quantitation (LOQ) were <1 ng/L for 22 of 24 compounds, being in compliance with the threshold criteria established by the EU Drinking Water Directive. Furthermore, multi-residue food safety methods have evolved to include pesticides, veterinary drugs, and mycotoxins in one 8-min UPLC gradient, assisting in reducing the overall laboratory cost of analysis per sample by an estimated 45 % when

compared to the individual analysis of these residue groups via HPLC analysis. (80)

Gap Analysis

Method Transferability and Standardisation Deficits

The lack of either an accepted universal data model to assist researchers with the applications of High-Performance Liquid Chromatography (HPLC) or Ultra-High-Pressure Liquid Chromatography (UHPLC) is one of the more significant unresolved issues in modern liquid chromatography. There are several existing geometric equations for a variety of chromatographic parameters such as flow-rate, column length, internal diameter, and particle size; however, these equations are only generally useful as of late, given the fact that most of the chromatographic stationary phases produced by different manufacturers exhibit considerable differences in selectivity and properties; i.e., completeness of end-capping, packing-density of bonded-phase, and pore-geometry. A recently published meta-analysis on 45 published method-transfer studies between 2020 and 2024, conducted by Vandenbosch and colleagues, indicates that only 58% of the method transfers were completed satisfactorily without further experimental troubleshooting. The remaining 42% of method transfers required adjustments of gradient slope, column temperature or mobile-phase additive concentration to complete satisfactorily, which represents the majority of method transfers conducted in FDA-approved laboratories that have to undergo partial or full revalidation in order to comply with applicable regulations or standards. (81-83)

The transferability gaps in testing procedures have been further exacerbated due to there being no internationally accepted standard for converting methods from HPLC to UPLC. In terms of analytical method development, ICH Q14 acknowledges the need for an approach to allow for flexibility in the analytical procedures, but does not state

any quantitative criteria for the acceptance of transfer performance. (84)

Pharmacopoeial monographs, including Ph Eur. and USP, specifically detail HPLC conditions and list 4.6 mm internal diameter with 5micron particle size columns, without the equivalent validated UPLC conditions; therefore, it is necessary for pharmaceutical testing laboratories to independently demonstrate equivalent performance of HPLC and UPLC methods on a resource-intensive basis, a major burden for small and medium-sized enterprises. (85)

Reproducibility and Inter-Laboratory Variability

HPLC/UPLC literature has a weakness in reproducibility that has been noticed in the literature by systematic reviewers across various applications. In an inter-laboratory study done in 2022 by the European Analytical Column Testing (EACT) consortium, 12 different laboratories analysed the same pharmaceutical reference mixtures using what were marketed as equivalent UPLC columns from a single vendor. For retention time, the relative standard deviations range from 0.8% (within laboratory) to 8.4% (between laboratories). For peak area reproducibility between laboratories, the most asymmetric peaks have a relative standard deviation that ranges from 4.2% to 18.7%, which exceeds the ICH Q2(R2) intermediate precision guidelines for pharmaceutical methods. The major drivers identified for the differences among laboratories were: instrument dwell volume differences (80–340 μ L among nominally equivalent instruments), temperature control precision differences (± 0.3 – 2.1°C), and differences in the mobile-phase preparation protocols employed. (86,87)

Environmental methods that use ultra-performance liquid chromatography retain similar issues with reproducibility as do other analytical methods; this can be seen in the results of a recent NORMAN Network collaborative trial to measure PFAS in surface water. In this collaborative study, 18 laboratories using ultra-performance liquid chromatography mass spectrometry (UPLC-MS/MS) from four instrument manufacturers generated quantitative results within a factor of two for only 14 of 24 PFAS congeners across all study sites. (88) When measured against one another, the inter-laboratory variation in results from short-chain PFAS (C4–C6) exhibited relative standard deviations greater than 50%, which was attributed to differences in sample preparation methods, sample preparation protocols, ion suppression management, and the lack of isotope-labelled internal standards for all analytes. These findings suggest that method harmonisation of environmentally available chromatography lags considerably behind that of the pharmaceutical industry, even though they have the same or even greater regulatory stakes. (89)

Cost and Accessibility Challenges

The capital expenditure differences between UPLC and HPLC systems are not as great as many think. The financial implications of using UPLC compared to HPLC, including the additional costs incurred by UPLC use in these low-resource environments with highly complex analytes, would ultimately be prohibitive. Therefore, while there may be capital expenditures associated with the acquisition

of an instrument or purchase of consumables, the larger issue of operational costs associated with consumables (e.g., column replacement) is likely to limit the cost-benefit of using UPLC in these types of laboratories. The use of sub-2- μ m particle columns for UPLC will result in column life spans of only 2,000 to 5,000 injections, while comparable HPLC columns are expected to last 10,000 to 15,000 injections; thus, UPLC is much more likely to be subjected to particulate and biological fouling than HPLC. In LMIC laboratories, the annual consumable costs associated with column replacement may account for 20% to 30% of their annual consumable budgets. The practical barriers to UPLC adoption that arise from these column lifecycle and replacement costs are largely absent from the published literature. (90,91)

The unequal opportunity for academic researchers to have access to an open-access instrument also creates an underappreciated gap. In 2023, a survey of more than 86 academic analytical chemistry departments across 22 different countries showed that 64% of these facilities did not have a UPLC-HRMS instrument and used HPLC-PDA as their source of research output. This structural difference results in the published literature being biased towards high-resource institutions and may explain the greater reliance on HPLC-based methodologies for the development of methods in LMICs, where they may be adequate but are clearly not leading-edge - a systemic divide that has a downstream effect within the global health and environmental monitoring context. (92)

Sensitivity and Selectivity Gaps

UPLC-MS/MS can measure concentrations of small molecules down to the nanogram/mL range when the sample does not contain many contaminants. However, detecting some small molecules will be much more challenging due to contamination levels and the lack of standard retention methods used by most analytical methods today. Substances that have low molecular weights (i.e., polar), are present at low concentrations (i.e., short-chain fatty acids, monosaccharides) and tend to ionize are not retained well on traditional "reverse phase" columns regardless of the particle size of the stationary phase, which necessitates using ion-pairing reagents or "HILIC" columns, both of which introduce additional selectivity issues and suppress ionization of the analyte by the mass spectrometer. Currently, the use of UPLC-MS/MS technology for quantifying endogenous compounds in situations where analytes may be isobarically interfered with (i.e., leucine/isoleucine, hydroxy-analogue of a drug coeluting with an endogenous steroid) requires using either bespoke chiral columns or HILIC columns, which significantly limits the ability to quantitate endogenous analytes on UPLC-MS/MS instruments generally. (93,94)

Simultaneously analysing structurally varying compound categories (e.g., Antibiotics, Hormones, Pesticides, PFAS) in one UPLC run is limited by the different chemical classes having incompatible ionisation polarities and fragmentation patterns; therefore, multiple methods have been required where a single method could have been used. Advancements in the development of true universal multi-class environmental screening methods have resulted in methods capable of covering between 150 and 200 analytes

in one run, but simultaneous detection of all low ng(ng/L) and the ability to measure each analyte at that level in the same run have not yet been accomplished, as the trade-off between sensitivity for each chemical class remains a barrier. (95)

Gaps in Green Chemistry Implementation

Between 2020 and 2025, the number of publications utilizing green chemistry metrics via HPLC/UPLC multiplied by three times as many; however, there are still considerable gaps in this trend. The majority of published articles reporting green methods typically restrict the environmental evaluation of their methods purely to mobile phase composition and don't take into account the use of solvents for sample prep or the energy required to synthesise reagents or power instruments. These are all included within the AGREE metric; yet authors tend to neglect them in order to achieve a higher score, as explained through the example in the references. Furthermore, multiple access to different green metrics (NEMI, EcoScale, GAPI, AGREE, COMPLEXE) has resulted in discrepancies when comparing methodologies used for evaluating greenness; thus, there were only 8% of papers published regarding green HPLC methods that utilised more than one green evaluation method as per the results provided in the 2024 systematic review. (96,97)

The label "water-rich" or "solvent-less" for mobile phases in reversed-phase HPLC is often only applicable to isocratic analyses of single compounds; therefore, the ability to generalise the effects of the green approaches reported here to high-throughput gradient applications is not confirmed when considering the range of polarities of analytes present in routine QC use in pharmaceuticals. (98)

Artificial Intelligence Integration: Premature Claims and Validation Gaps

While machine learning is being rapidly incorporated into the development of chromatographic methods, there is currently a significant validation deficit associated with these applications. Most existing machine learning models have been tested and trained with data generated at a single lab, from a single instrument platform, or through the use of structurally predetermined compound libraries which limits the ability to generalise those models. (99) There are no studies published between the years 2020 and 2026 that have demonstrated prospective, multi-laboratory validation of an ML-enhanced HPLC/UPLC method development system against the ICH Q2(R2) validation criteria. The lack of this type of validation is a major shortcoming if institutions wish to pursue the regulatory adoption of such methods as called for by the ICH Q14 for lifecycle management of analytical procedures. (100)

Future Directions

Standardisation and Regulatory Harmonisation

The development of a universal method conversion toolkit from HPLC to UPLC is an area that could have the single largest short-term impact on the chromatographic community, as well as developing a pharmacopoeial guidance document. This toolkit should provide algorithms for matching the relative selectivity of columns based on hydrophobicity subtraction model parameters, dwell

volume correction factors and validated gradient transfer calculations between at least 20 laboratories and four major instrument platforms. The USP Chromatography Expert Committee and EDQM's General Chapter Working Group are logical representative institutions for this project, as well as engaging with instrument manufacturers to standardise dwell volume characterisation protocols.

AI and Machine Learning: Toward Regulatory- Ready Models

For ML-assisted technique development to be accepted by authorities, there are three concurrent areas of research required: (i) the creation of extensive publicly available databases with retention time information for varied compounds in the pharmaceutical and environmental sectors across various stationary phases—similar to the Report database used for predicting retention time in reverse phase chromatography; (ii) the systematic validation (i.e. prospective validation) of model generated gradient optimization tools to meet the ICH Q2(R2) guiding criteria through multi-laboratory based studies; and (iii) inclusion in any publication reporting ML related advancements in method development of the relevant XAI output (e.g. SHAP values reflecting the contribution of any given set of physicochemical descriptors towards the predicted retentions of an analyte) in order to provide a stringer evidential basis to support regulatory submission for methods developed using ML. These requirements complement the evolving structure of Analytical Procedure Lifecycle Management outlined in ICH Q14 while also providing robust evidence to support regulatory submission for methods developed using ML principles.

Integration with Nanotechnology

Nanoparticle stationary phases (including silica nanoparticles modified with molecularly imprinted polymers, or MIPs; carbon nanotubes; and graphene oxide) have been shown to have selectivity profiles that cannot be matched by typical bonded silica phases. Currently, the biggest barrier to commercial acceptance of MIP-based high-performance liquid chromatography (HPLC) is reproducibility; variations in template removal efficiencies from batch to batch lead to analyte-specific response factors that may differ by 10–40% between columns produced from the same synthesis batch. Improvement of sol-gel encapsulation techniques and microwave-assisted MIP synthesis have been reported from 2022 to 2025; however, before MIP-HPLC can be considered for regulated uses, significant efforts will need to be made in systematic improvement and independent confirmation studies.

Microfluidics and Lab-on-Chip Chromatography

The integration of UPLC with microfluidic sample processing systems—combining on-chip solid-phase extraction (SPE), protein precipitation (PP), and column-switching with integrated UPLC separation—represents a potentially significant advancement towards point-of-care clinical analytics. Existing chip-LC platforms have demonstrated enough sensitivity to allow for therapeutic drug monitoring using dried blood spots; however, to develop microfluidic interfaces that will work universally across the diverse set of clinical matrices (urine, saliva,

cerebrospinal fluid) and analytes needed for clinical decision-making presents a currently unmet research need. Investment in photolithographic fabrication of columns with below 1- μm packed beds, which has already been demonstrated at the lab level, may create chip-UPLC instruments that are capable of achieving 100,000 plates/m at less than 200 bar pressures—appropriate for use with portable platforms using syringe pumps.

Discussion

Synthesis of Findings

Between 2020 and 2026, there have been incremental but cumulatively significant developments in all the key technologies related to HPLC and UPLC. The gradual evolution of column technology, particularly the maturing of SPP or small particle porous phase materials, as well as the broadening of the number of options available for the column stationary phase materials, has enabled both platforms to operate in an expanded operational window without fundamentally altering the design of the instrumentation being used. The introduction of improvements to the sensitivity of detectors, particularly about triple-quadrupole and high-resolution mass spectrometry detector platforms, has not only improved the ability of UPLC-MS/MS methodology to obtain quantitative data, but also increased the number of applications that UPLC-MS/MS methodology can cover that were previously only reachable through immunoassays or radiometric methodologies. The emerging formalisation of green analytical chemistry and the introduction of established criteria for evaluating the sustainability of laboratory methods have created a common and standard set of terminology for measuring sustainability that represents considerable advancement from the unstandardised solvent accounting before the introduction of the newer terminology, but has not yet become large enough to encompass all of the multiple metrics that have recently emerged.

From reading through the literature, we see a clear trend in UPLC hardware becoming more and more similar to the data science tools. A distinct shift from using trial and error type experiments to developing ML-assisted method development can be observed across pharmaceutical, environmental and bioanalytical literature; however, they are still all very new and not yet fully validated. Based on multiple independent research groups coming to a consensus, ML models can predict retention and are useful as a means of narrowing down on the method development; however, experimental confirmation and regulatory validation cannot yet be replaced, which is a more subtle point that some of the publications promoting the technology do not convey well.

Trends and Emerging Consensus

There are actually several areas of true and emerging consensus amongst these authors. The standard stationary phases for UPLC-pharma, the sub-2 μm BEH and CSH C18 columns, have gained the primary spot since there are numerous studies showing that these columns have improved selectivity over all other commercially available stationary phases. A major change in the use of instrumental methods occurred as UPLC-MS/MS has taken

over as the primary method (instead of HPLC-UV) for regulated bioanalysis by the vast majority of laboratories meeting the most stringent regulatory requirements, mainly as a result of new guidance documents from both the EMA and FDA providing criteria for making regulatory submissions to be made via MS detection. Another emerging consensus is that the AGREE index will likely become the only routinely used metric to use as an indicator of the 'green' nature of methods published since it has been cited in approximately 180 peer-reviewed articles from 2021 to 2025 — an unprecedented rate compared to all previously published green metrics.

Conflicting Evidence and Unresolved Debates

The gap analysis has highlighted many inconsistencies in published research, but there are two overriding issues that have yet to be resolved within the literature. The first is related to particle architecture for gradient separations. Theoretical equivalent kinetic performance of SPPs and sub-2 μm fully porous particles has yet to provide consistent equivalent practical performance across the full range of gradient separation conditions; further research is necessary to determine the resolution of this contention (A coordinated multi-laboratory study, using harmonised gradient protocols, is necessary.) to achieve a consensus on the appropriate particle architecture to be used for gradient separations.

The second issue that has yet to be resolved is the appropriate placement of HPLC versus UPLC within pharmacopoeial procedures. UPLC advocates claim that it is now sufficiently mature to be implemented in standardised methods, while members of the conservative faction claim that UPLC cannot achieve the necessary degree of reproducibility when assessed against UPLC and HPLC performance criteria due to the lack of standardisation between instruments, which, in turn, undermines the reproducibility standards that are essential to compendial methods.

The potential for development papers that employ ML-based methodologies also highlights the challenges associated with the need for comprehensive data reporting standards. There is an enormous variability in the way publication authors describe their model(s), define their training and testing datasets (transparently), and report performance metrics, rendering apples-to-apples comparisons among various published studies virtually impossible. The implementation of reporting standards like CONSORT (Clinical Trials) or PRISMA (Systematic Review) specifically for ML-based studies published in analytical chemistry journals would provide a significant level of evidential quality enhancement to this growing body of published literature.

CONCLUSION

The current review has systematically analysed the scientific advancements in HPLC/UPLC technology over the period between 2020 to 2026. The review highlights advances in column technology, novel detectors, environmentally sustainable chemistry, automation, and artificial intelligence, and provides a critical evaluation of any gaps in current knowledge as well as a plan for future research.

The key findings from this review are as follows:

Significant advancement in UPLC technology has occurred to date, and will continue to unfold rapidly, with the development of sub-2 μm SPP columns, UPLC-HRMS systems, and the application of machine learning to facilitate method development representing the three most transformative advances during this time period. The above-mentioned developments significantly improve the sensitivity, speed, and sustainability of liquid chromatography across the pharmaceutical, environmental, food safety, and bioanalytical disciplines.

However, the gap analysis conducted in this review demonstrates that the majority of the research community has not been as successful at developing new methods as they have at properly assessing and documenting their limitations. This analysis has identified the following as the most pressing research priorities: (i) developing a unique HPLC to UPLC transfer toolkit and validating it with multiple laboratories; (ii) creating an open access retention database for use in allowing ML (machine learning) models to be used across multiple labs; (iii) unifying all aspects of green metric reporting by developing one validated reporting framework; (iv) extending validated UPLC methods to areas with no current application (i.e., RNA therapeutics, continuous manufacturing by utilizing the PAT [process analytical technology] concept, and field deployable environmental monitoring).

While HPLC and UPLC will continue to serve as critical analytical tools in the coming years, the analytical community must aggressively address identified gaps to fully realise the value of these tools in a world with more complex samples, stricter regulations, and higher sustainability responsibilities than ever before.

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