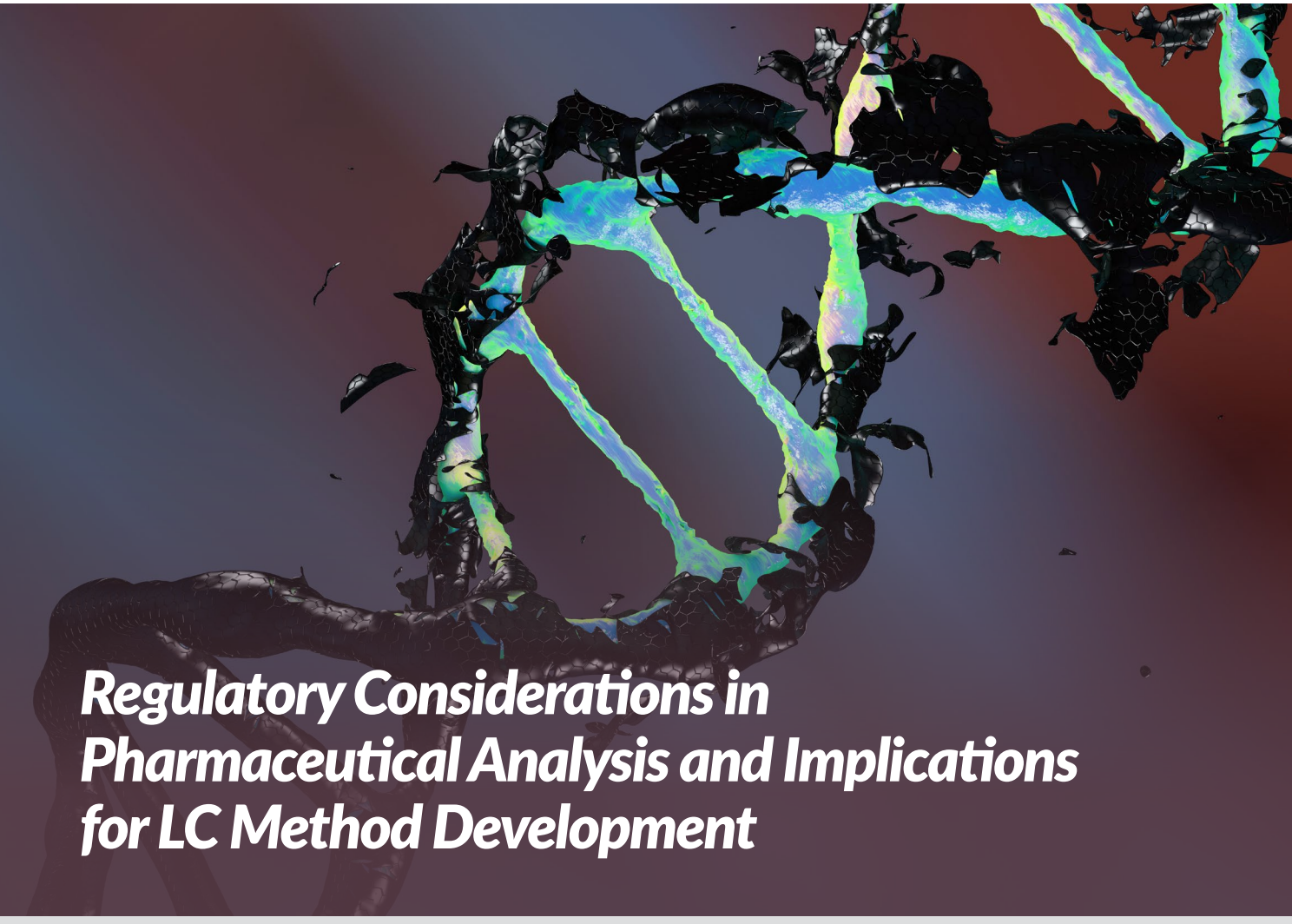
LCGC®

FEBRUARY 2022

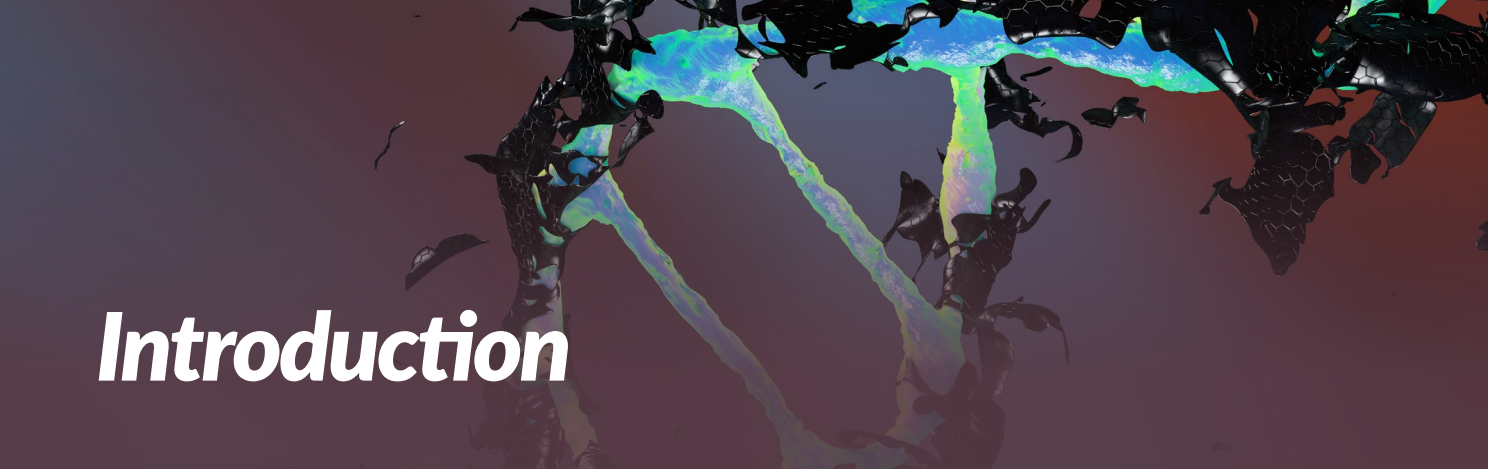


Regulatory Considerations in Pharmaceutical Analysis and Implications for LC Method Development

- Establishing the FDA
- Establishing the International Council for Harmonisation (ICH)
- Quality By Design (QbD) in LC Method Development
- Examples of Achieving Regulatory Requirements in GC and LC analysis

This custom ebook is sponsored by Avantor and presented in partnership with *LCGC*.

avantor™



Introduction

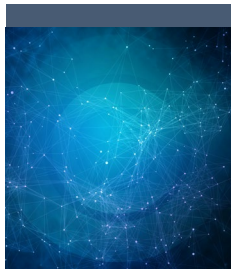
History of Regulation in the Pharmaceutical Industry

The development of the pharmaceutical industry has seen substantial changes in the way it is regulated. The U.S. Food and Drug Administration (FDA) is a prime example of how the regulator has grown from one individual investigating the adulteration of food products to an organization employing nearly 18,000 employees with a budget that exceeds $5 billion. (1) The increase in regulatory power has resulted in the production of safer drug products and more ethical approaches to their development and manufacture, resulting in very significant changes in the way drugs are advertised and tested. It is evident that, through the development of the stature of the regulatory authorities, chemical analysis has become an essential tool in monitoring the quality and safety of manufactured drug products.


"Poison Squad" Volunteers | Image courtesy of FDA, flickr.com

Establishing the FDA

Initially one individual, Charles M. Wetherill, was appointed by President Lincoln to serve as the first chemist in the new U.S. Department of Agriculture. (2) This was the beginning of the Bureau of Chemistry, the predecessor of the FDA. Initially, the task was overwhelming, as adulteration was rife, and the approach that was being taken was not objective enough. Thus, the Commissioner of Agriculture, George Loring, in 1882 replaced Peter Collier, his current chief chemist, with someone who could employ a more objective approach. Harvey Washington Wiley, who had a practical knowledge of agriculture, a sympathetic approach to the problems of the agricultural industry, and an untapped talent for public relations, took the role on with vigour. The division expanded rapidly and also introduced poison squads, whose role was to test deliberately adulterated food to determine the impact that various illegal additives would have. In 1906, President Theodore Roosevelt issued the Pure Food and Drugs Act, which was effectively written by Wiley. (3) Wiley started to use the new powers to aggressively route out



wrongdoers, but unfortunately, was deemed to be overzealous in his approaches by a judicial review. This left a series of potentially dangerous marketed products that were not covered by legislation.

By the 1930s, a campaign was launched for stronger regulatory authority by publicizing a list of dangerous products that had been ruled permissible under the 1906 law. The list of products is incredulous when applying modern standards and included:

- Radioactive beverages
- A mascara that caused blindness
- Cures for diabetes and tuberculosis that did not work

Unfortunately, the legislation generated due to this interest struggled to get through the United States Congress, and it needed a major incident to turn public opinion and Congress to allow the bill to pass. In 1937, a Tennessee drug company, S.E. Massengill Co., began marketing a product called Elixir Sulfanilamide. (4) Sulfanilamide is a drug used to treat streptococcal infections and had been shown to be effective and was safely used for some time in both tablet and powder form. In June 1937, a salesman reported demand for the drug in liquid form, and so the company's chief chemist and pharmacist, Harold Cole Watkins, devised a new formulation. The new formulation used diethylene glycol to dissolve the sulfanilamide but was not tested for toxicity because safety studies were not required for new formulations. Diethylene glycol is commonly used as antifreeze and is a deadly poison. This new formulation resulted in over 100 deaths, mainly children since it was seen as an easier method for administering the drug than powder or tablet formulations. (5)

The resulting public outcry over new drugs not needing safety testing meant that President Franklin Delano Roosevelt was able to sign the Federal Food, Drug, and Cosmetic Act, which came into law on June 24, 1938. (6) The new law significantly increased federal regulatory authority over drugs by mandating a pre-market review of the safety of all new drugs. False therapeutic claims in drug labeling were banned, and animal testing was introduced as part of the drug development process to ensure safety. It is noteworthy that the legislation introduced in the United States was far tighter than in other countries,

Image courtesy of FDA, flickr.com

and consequently, the United States did not suffer the issues associated with the morning sickness drug, thalidomide, that beset many other countries. (7) By 1962, laws were passed that allowed for the inspection of drug manufacturing plants and tighter controls on the manufacture and approval of drugs. (8)

GLP & cGMP

Over the following decades, numerous and infamous incidents—for example, those involving Industrial BioTest Lab (IBT) and G.D. Searle & Co.—highlighted some of the abhorrent practices that were employed in animal-testing facilities. (9,10) In 1977, IBT was criminally implicated for producing fraudulent studies on a range of products, including Nemacur, Sencor, Naprosyn, and trichlorocarbanilide. (9,11) The FDA audit determined that 618 of 867 (71%) studies performed by IBT were invalidated for having "numerous discrepancies between the study conduct and data." (12,13) Although the findings were confidential, some of the practices were leaked, and these included poor or inadequate record keeping, which invalidated test results. In a number of cases, sick test animals were replaced with healthy ones, invalidating any test results produced. (14) Three former officials of the company were eventually convicted after one of the longest trials in U.S. history of fabricating key product safety tests. (15)

These cases very much instigated the concept of Good Laboratory Practice (GLP), which was introduced into the United States, although other countries had introduced this concept before, most notably New Zealand. Six-hundred new inspectors were hired to ensure that the new standards were being met by the industry. GLP aims to ensure the integrity of non-clinical laboratory studies through planning, performing, monitoring, recording, archiving, and reporting. This was very much an era of quality-by-inspection, which was the prevailing philosophy for another 20 years. More recent decades have seen a change in direction by the regulators, who directed the industry to move toward quality by design, where a greater emphasis is placed on a better understanding of the process. Although it is worth noting that the principles of GLP define a set of rules and criteria for a quality system, it does not, per se, assume that the best scientific approach will be employed.

GLP is one facet of a series of systems designed to control quality within the pharmaceutical industry. As well as GLP, GCP (Good Clinical Practices) and cGMP (current Good Manufacturing Processes) exist. The scope for cGMP also covers laboratory practices related to the quality-control procedures for intermediate and final products produced within the manufacturing environment at a pharmaceutical plant. These quality systems are also used in other industries such as food and beverages, cosmetics, and medical devices. These practices are driven by regulatory authorities, to improve the quality standards associated with these industries. The regulatory authorities are driven to a certain extent by the events in these industries, such as the thalidomide event mentioned previously.

avantor™

Equipment Services

Keep your laboratory in compliance and in operation

Providing a carefree equipment lifecycle with Avantor Services

- Installation
- Preventive maintenance
- Warranty and repair
- Calibration
- Qualification, validation and certification (IQ/OQ/PQ)
- Equipment management
- Training

avantorsciences.com/equipment-services

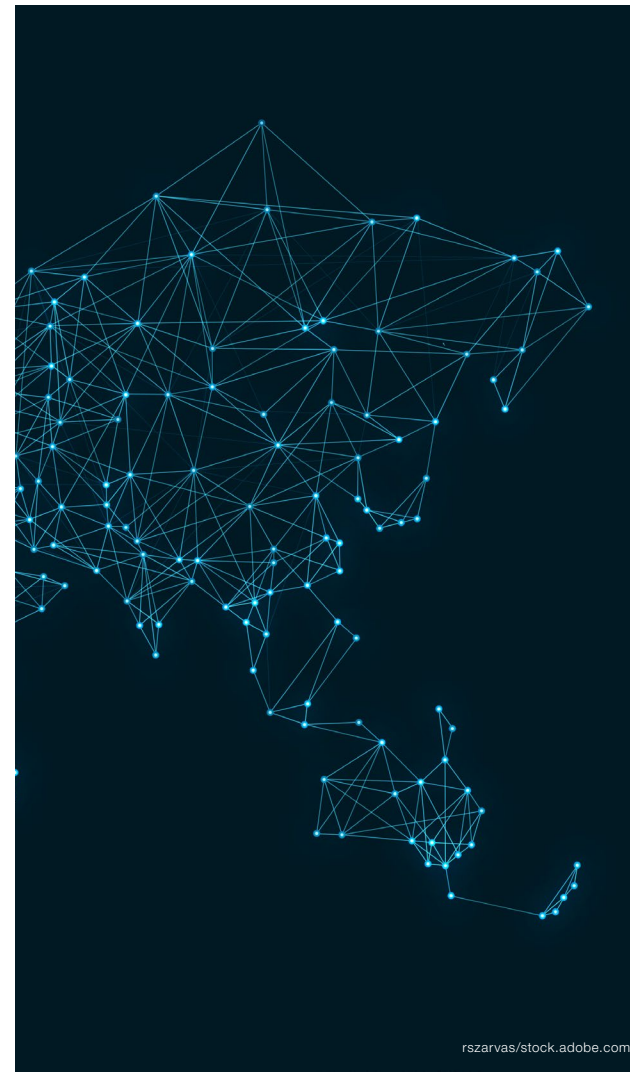
Establishing the International Council for Harmonisation (ICH)

The thalidomide incident (7) highlighted the need for harmonization of the different regulators from a safety perspective; however, the industry was also finding that a lack of harmonization resulted in a great degree of duplication in addition to time-consuming and expensive testing procedures, which did not add value to the product or increase consumer safety. This was, in part, driven by a substantial increase in the legislation throughout the 1960s and 70s, relating to the reporting and evaluating of data on the safety, quality, and efficacy of new medicinal products, which generated a divergence in the technical requirements from one country to another. This increase in administration and laboratory effort resulted in drugs being delayed and substantial increases in the costs of the drugs being sold.

Harmonization of regulatory requirements was pioneered by the European regulators under the guise of the newly formed European Community (EC). In the 1980s, Europe moved toward

ESTABLISHING THE INTERNATIONAL COUNCIL FOR HARMONIZSATION (ICH)

the development of a single market for pharmaceuticals. The success achieved in Europe showed that harmonization of the regulations was feasible; and at the WHO (World Health Organization) Conference of Drug Regulatory Authorities (ICDRA) in Paris in 1989, specific plans for the formation of a common set of standards began. The birth of ICH took place at a meeting in April 1990 in Brussels, where representatives of the regulatory agencies and industry associations of Europe, Japan, and the United States met. (16) Since this meeting, the ICH process has gradually evolved with significant progress being made in the development of ICH Guidelines on Safety, Quality, and Efficacy topics in the first decade. Beyond this timeframe, the ICH has looked to expand its reach into other regions, ensuring a common set of standards can be applied. This has had a significant impact in reducing the costs of drugs while ensuring that new approaches can be implemented in a timely manner. This has been applied to the analysis of impurities within the drug production process.



avantor™

Find out more at
vwr.com/
chromatography

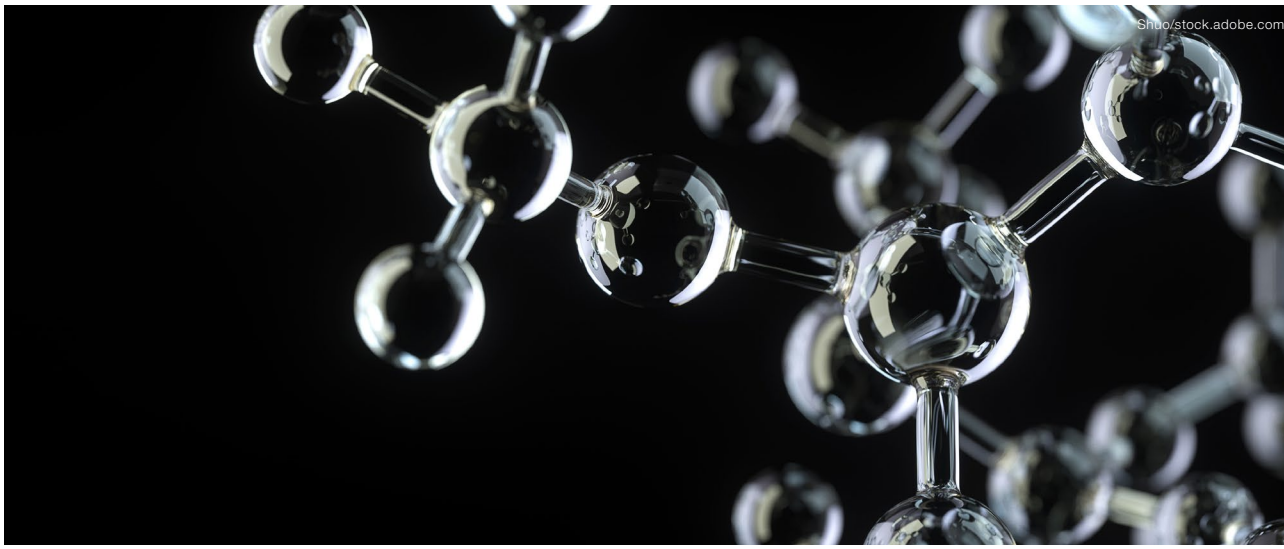
Automated UHPLC method development solutions with novel stationary phases

Solve HPLC method development challenges systematically and efficiently – using Avantor® ACE® method development kits – providing chromatographers with more choices for alternative selectivity, without compromising stability or robustness. Combined with ChromSword Auto® 5 Hitachi Edition and VWR Hitachi ChomasterUltra Rs, maximise productivity in your method development processes.

VISIT OUR VWR CHROMATOGRAPHY SOLUTIONS WEBSITE

- Product pages, application areas and product links
- Knowledge centre: Videos, white papers, technical papers
- Application library: Search for thousands of applications

vwr.com/chromatography
vwr.com/ace



Quality by Design (QbD) in LC Method Development

The ICH has been instrumental in driving new ideas and approaches to how regulators and the pharmaceutical industry interact. Until recently, the approach that many pharmaceutical companies applied for developing and validating an assay would be to ensure that the assay complied with the guidance stipulated by the regulators. However, this approach does not encourage the industry to look at better methods of testing. Consequently, ICH launched guidelines (ICH Q14) that seek to address this situation. (17) Specifically, ICH Q14 looks to address the area of assay development since there was no ICH guideline on analytical procedure development. ICH Q14 is based on the concept of QbD, where the robustness of the analytical method is evaluated as part of the method development process. It also inherently encourages scientists to look at approaches to making the analysis better, and this includes the use of innovative technologies such as automated method development, screening platforms, and retention modeling software. This acceptance that innovative technologies should be

actively encouraged is an exciting prospect for scientists within the pharmaceutical community but also for the manufacturing world, which has often struggled to move new ideas into the highly regulated environment of the pharmaceutical industry.

The concept of QbD impacts liquid chromatography (LC) method development, where it is feasible to optimize a method by varying specific method parameters, such as flow rate, mobile phase composition, etc., using a multi-factorial approach. This approach can be readily incorporated into the method development process and the use of DoE (Design of Experiments) ensures that robustness is inherently built into the analytical. A full list of parameters that can be investigated would include:

- Stationary phase chemistry
- Mobile phase composition
- pH of mobile phase
- Column temperature
- Gradient profile
- System dwell volume
- Flow rate
- Column batches

Ultimately, this process of method development leads to the generation of a robust method that can be validated. Validation is the process by which it is established, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended purpose. For the pharmaceutical industry, the method validation requirements relate the linearity, specificity, selectivity, accuracy, and precision, which have to meet the requirements stipulated by the regulatory authorities.

RESOURCES:

- Systematic Reversed-Phase Method Development Protocol Using ACE MDKs
- A Simple Step-by-Step Protocol for HILIC Method Development
- An Intelligent Solution for Hydrophilic Interaction Liquid Chromatography Method Development
- Transferring LC Methods Between Different Instruments Using the Avantor® ACE® LC Translator Tool

Stationary Phase Characterization

It is well established that high-performance liquid chromatography (HPLC) columns are not all the same, and indeed the same nominal stationary phase packed in a column does not necessarily result in the same retention times or even the same elution order. (18,19) Understanding the retention mechanisms that define chromatographic separations is a critical component of developing methods to separate an active pharmaceutical ingredient (API) from excipients and potential impurities. The understanding of retention mechanisms has evolved dramatically in recent decades, with pivotal work being performed by Tanaka and also Euerby in terms of column characterization. (20,21,22) The understanding of retention mechanisms for reversed-phase columns was initiated by the work of Tanaka, (21) who devised a series of test probes and test conditions that would quantify specific retention mechanisms. The initial work defined a series of relatively simple probes that assessed:

- Hydrophobicity
- Hydrophobic selectivity
- Degree of hydrogen bonding
- Ion exchange capacity at a pH above and below the nominal pK_a of silica

This work has been supported by the development of relatively simplistic retention models, together with use of molecular mechanics to model specific interactions. With the latter, the increase in computational power has resulted in the ability to model not just the interaction between a stationary phase molecule and a compound but also investigate solvent effects. (23)

This work has been extended greatly by a number of researchers. As such, the number of potential probes far exceeds 100, and most modes of HPLC are now covered. The one area that is lacking is the retention modeling of large molecules such as proteins; however, even here early steps are being taken. (24) This approach is something that regulators are also looking at. The introduction of the United States Pharmacopeia (USP) website that specifically shares characterization information on 700+ columns shows the level of commitment to this approach to aiding separation scientists in developing their methods. (25)

RESOURCES:

- The Power of Stationary Phase Selectivity
- Analyte Properties: pKa, logP, and logD
- Maximization of Selectivity in Reversed-Phase Liquid Chromatographic Method Development Strategies
- Webinar: Method Development Strategies Using Selectivity as a Tool

Mobile Phase

Column selection is an important parameter for LC; however, other experimental parameters can also influence the retention

and separation of analytes. In addition to the consideration of the stationary phase, the mobile phase also has to be considered, as it can also have a substantial effect on the retention time of individual analytes, as well as separation selectivity. It has been shown that the use of electron-donating solvents, such as methanol, will have a different selectivity to dipole solvents such as acetonitrile. (26) In addition, there is a clear relationship between retention time and the amount of organic solvent present in the mobile phase. Thus, an important consideration when looking at the stability of the assay is the composition of the solvent, the rate of change of the solvent composition, and the delivery flow rate, in particular with non-isocratic systems where the flow of one of the channels may be significantly lower than the recommended flow for the pump that is being used.

RESOURCES:

- Considerations and Best Practice for Mobile Phase Buffer Selection and pH Control for LC and LC-MS Method Development

pH Effects

One of the other parameters associated with the mobile phase is the pH. Since a large proportion of analytes have acidic or basic properties, the pH will influence the charge state of the molecule. The log distribution (logD) will define the hydrophobicity of a molecule as the pH is altered, with acids being proton donors, existing in a charged state at higher pHs, and bases, defined as proton acceptors, having a charged state at lower pH values. The charged form of the molecule will have less hydrophobicity, logD, and hence will retain less on a standard reversed-phase column. The transition pH where this occurs is around the pK_a and typically this occurs ±2 pH units around the pK_a. In general, it is advisable not to perform separations around the pK_a as it creates a large degree of variability in retention time and peak shape.

A buffer is typically used to ensure that a stable pH is maintained on the column. It is important to ensure that the buffer has a suitable working pH range, as this will have an impact on the retention time stability. Additionally, buffer concentration should be assessed, as too low a concentration may not be effective in maintaining retention-time stability.

RESOURCES:

- Use of Mobile Phase pH as a Method Development Tool

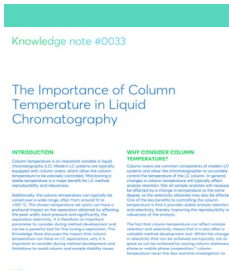
Temperature

Temperature is often used in some forms of chromatography as a key variable in method development, in particular, gas chromatography (GC); however, it is seldom used in HPLC. It can, however, be incredibly useful for optimizing a separation, and so having a thorough understanding of the impact that changing the temperature has is important. There are several key considerations when assessing the operating temperature:

- Column stability
- Compound stability
- Effect on peak width
- Effect on retention time

RESOURCES:

- Achieving Superior Separations with Innovative Avantor® ACE® Chromatography Technology
- Chromatographic Band Broadening and the van Deemter Equation


Knowledge note #0033

The Importance of Column Temperature in Liquid Chromatography

INTRODUCTION

WHY CONSIDER COLUMN TEMPERATURE?

SPONSORED CONTENT

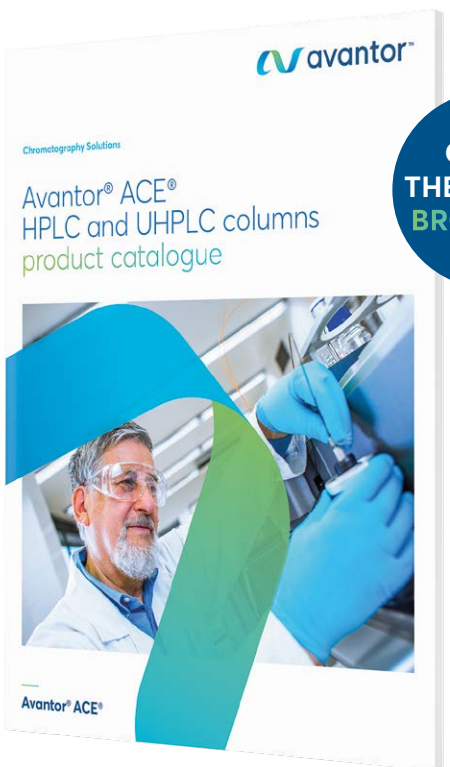
The Importance of Column Temperature in Liquid Chromatography

avantor™

Our new Avantor® ACE® U/HPLC columns catalogue is here!

Our Avantor® ACE® range is the flagship range of our high quality U/HPLC columns. This new catalogue highlights the technical advantages and different phases including:

- 20 phases for all types of applications
- Useful information showing what makes our Avantor® ACE® range the premium range on the market
- Novel phases such as the C18-AR or C18-PFP that are part of our method development kits
- Columns for HILIC applications
- 300 Å columns for proteins and peptides, and our new Oligo and Glycan columns
- Applications highlighting the best features of these columns

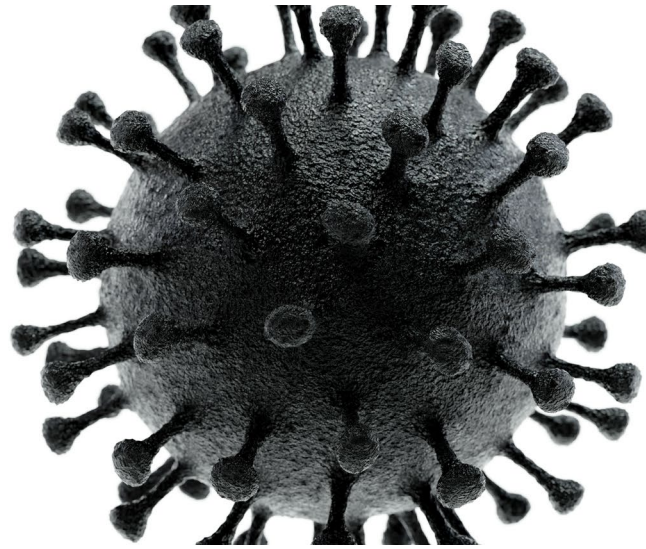


GO TO THE ONLINE BROCHURE

Empowering discovery, development and routine analysis through cutting-edge chromatography solutions

Avantor® ACE®
ULTRA-INERT BASE DEACTIVATED COLUMNS

Learn more at vwr.com/ace



Nader-T/stock.adobe.com

Examples of Achieving Regulatory Requirements in GC and LC analysis

Nitrosamines

In recent years, the determination of nitrosamine impurities in drug substances and products has become a high-profile focus point in the pharmaceutical industry. Nitrosamines are mutagenic and have been widely reported as occurring in a range of sources, including environmental samples and processed food products. Following the detection of NDMA in valsartan in 2018, the regulatory landscape has evolved rapidly, with the various authorities specifying a number of key nitrosamines of concern and applying strict, extremely low-level, allowable limits on their content in pharmaceutical materials.

The analysis of these compounds, therefore, requires the application of highly sensitive analytical techniques and typically employs either LC or GC in combination with mass spectrometry (MS). Additionally, the hydrophilic nature and low molecular mass of some nitrosamines mean that achieving adequate retention by reversed phase, and resolution from other isobaric, low-molecular-weight species is challenging.

The linked articles examine how the requirements of the regulatory authorities can be met by LC-MS/MS and how quantification accuracy can be improved through consideration and the use of alternative LC stationary phase selectivities.

RESOURCES:

- Low-Level Determination of Mutagenic Nitrosamine Impurities in Drug Substances by LCMS/MS
- Determination of Nitrosamine Impurities in Pharmaceutical API's and Addressing Issues Associated with DMF and NDMA Co-elution

COVID Drugs

The rapid outbreak of the SARS-CoV-2 virus in late 2019, leading to a pandemic by March 2020, necessitated the demand for effective pharmaceutical treatments to be rapidly identified and made readily available. Due to the inherently long timeframes associated with pharmaceutical development, and with health services being inundated globally, the industry sought to repurpose pre-approved drugs in the market to swiftly combat COVID-19. This approach utilized pre-existing clinical trial data available for meta-analysis, in addition to benefiting from the infrastructure already in place to supply the market with the pre-approved pharmaceuticals.

Rapid turnaround times for the development of analytical methods for pharmaceuticals are achievable through the diverse range of selectivities available via the Avantor® ACE® HPLC/UHPLC column range. Using two of the Avantor® ACE® Method Development Kits (MDKs), up to six stationary phases can be assessed. When combined with a simple protocol using two different organic modifiers (methanol and acetonitrile), a set of 12 chromatograms can be produced to guide the analyst toward the ideal set of conditions for their separation.

Impurity Testing

Stationary phase selectivity is a powerful tool for developing methods for impurity testing to ensure resolution and quantification of all impurity peaks. In the example shown in the resource box, the Avantor® ACE® C18-PFP was found to resolve impurity peaks for Favipiravir.

The Avantor® ACE® C18-PFP column can also be applied for the testing of Remdesivir. This method is now specified in the Indian Pharmacopoeia (IP) in methods for both assay of Remdesivir and the analysis of Remdesivir and related substances.

RESOURCES:

- Separation of Favipiravir and Impurities
- IP Method for Analysis of Remdesivir Related Substances
- IP Method for Assay of Remdesivir

Residual Solvents

As part of the manufacturing process, a variety of solvents will be used but not all will be removed from the manufacturing process, so a quality check is performed to ensure that the levels of solvents within the final material are at an acceptable level. This ensures that it does not impact the toxicity or efficacy of the formulation. The list of solvents used in drug manufacturing is commonplace and is displayed in TABLES 1 - 3. (27) It is generally accepted that the chromatographic method of choice for analyzing these types of compounds is GC. The different classifications of solvents are:

- Class 1 solvent (TABLE 1). Should not be used.
- Class 2 residual solvents (TABLE 2). Should be limited in drug substances, excipients, and drug products.
- Class 3 residual solvents (TABLE 3). May be regarded as less toxic and of lower risk to human health than Class 1 and Class 2.

TABLE 1: Class 1 Residual Solvents

Solvent	Concentration limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

TABLE 2: Class 2 Residual Solvents

Solvent	Permitted Daily Exposure (mg/day)	Concentration Limit (ppm)*
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890

* Calculated assuming 10 g product weight administered daily

TABLE 3: Class 3 Residual Solvents

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
tert-Butylmethyl ether	Methylisobutylketone
Cumene 2-Methyl-l-propanol	Pentane
Dimethyl sulfoxide	1-Pentanol
Ethanol	1-Propanol
Ethyl acetate	2-Propanol
Ethyl ether	Propyl acetate
Ethyl formate	
Formic acid	

This has resulted in a series of standardized methodologies that organizations use for the analysis of residual solvents within the final formulation.

RESOURCES:

- Separation of Residual Solvents (Class 1 Mix) Using HI-624
- Separation of Residual Solvents (Class 2 Mix A) Using HI-624
- Separation of Residual Solvents (Class 2 Mix B) Using HI-624
- Troubleshooting Methods in Gas Chromatography

avantor™

See the difference in chromatographic efficiency with our NEW Avantor® ACE® UltraCore solid-core U/HPLC columns

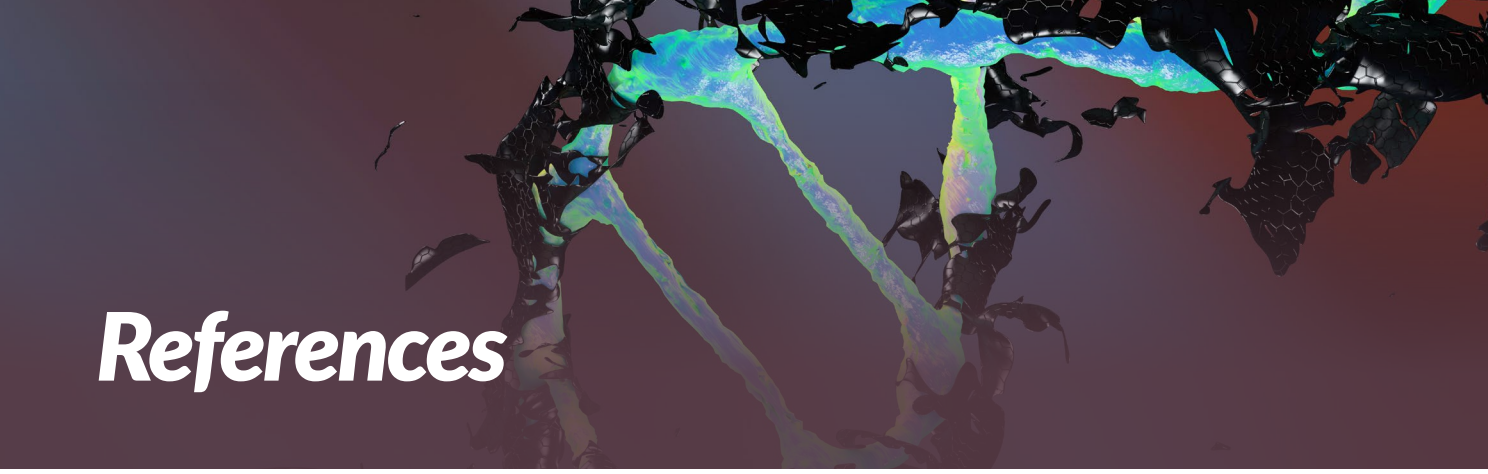
High throughput, high efficiency ultra-fast separations are achievable using Avantor® ACE® UltraCore - ultra-inert solid core (core shell) columns. Avantor® ACE® UltraCore columns utilise ultra-high purity solid core silica with a mono disperse particle distribution to combine high efficiency with low back pressure. Achieve UHPLC-like performance using HPLC instrumentation with Avantor® ACE® UltraCore.

GO TO THE ONLINE BROCHURE

Empowering discovery, development and routine analysis through cutting-edge chromatography solutions

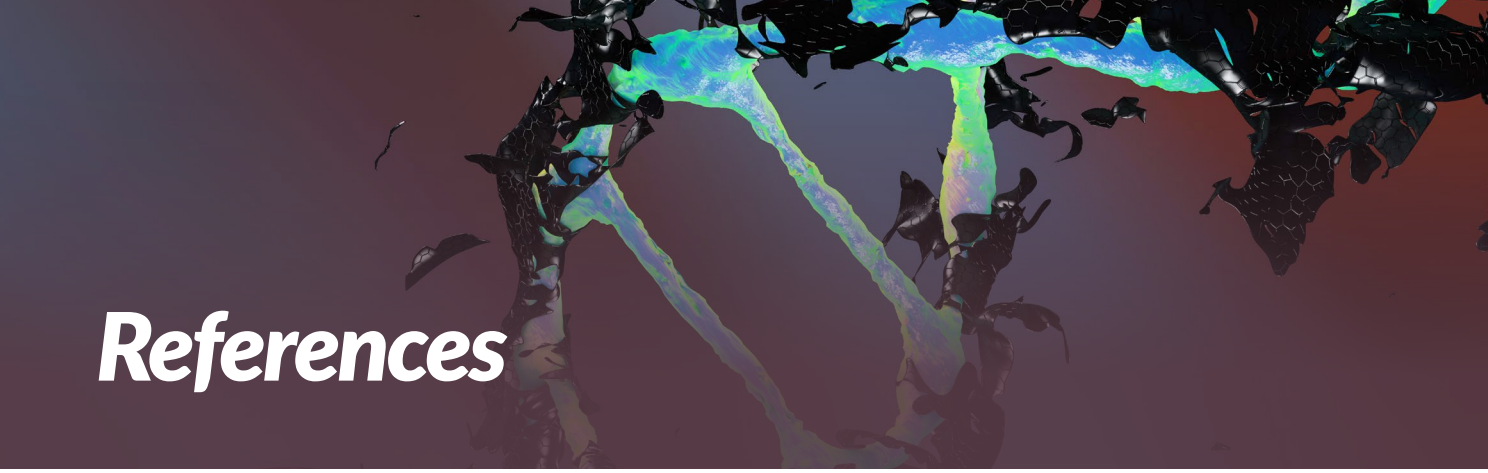
Avantor® ACE®
ULTRA-INERT BASE DEACTIVATED COLUMNS

Learn more at **vwr.com/ace**



References

1. FDA, “Fact Sheet: FDA at a Glance,” FDA, November 2020. [Online]. Available: https://www.fda.gov/about-fda/fda-basics/fact-sheet-fda-glance.
2. M. A. Hickman, The Food and Drugs Adminsitration (FDA), Nova Publishers, 2003.
3. “Part I: The 1906 Food and Drugs Act and Its Enforcement,” April 2019. [Online]. Available: https://www.fda.gov/about-fda/changes-science-law-and-regulatory-authorities/part-i-1906-food-and-drugs-act-and-its-enforcement.
4. C. Ballentine, “Sulfanilamide Disaster,” FDA, June 1981. [Online]. Available: https://www.fda.gov/files/about%20fda/published/The-Sulfanilamide-Disaster.pdf. [Accessed January 2022].
5. J. G. West, “The Accidental Poison That Founded the Modern FDA,” January 2018. [Online]. Available: https://www.theatlantic.com/technology/archive/2018/01/the-accidental-poison-that-founded-the-modern-fda/550574/.
6. “Federal Food, Drug, and Cosmetic Act (FD&C Act),” FDA, March 2018. [Online]. Available: https://www.fda.gov/regulatory-information/laws-enforced-fda/federal-food-drug-and-cosmetic-act-fdc-act
7. J. H. Kim and A. R. Scialli, “Thalidomide: the tragedy of birth defects and the effective treatment of disease,” *Toxicol. Sci.*, vol. 122, no. 1, pp. 1-6, 2011.
8. J. A. Greene and S. H. Podolsky, “Reform, Regulation, and Pharmaceuticals — The Kefauver–Harris Amendments at 50,” *N Engl J Med*, vol. 367, no. 16, pp. 1481-1483, 2012.
9. K. Schneider, “• Faking It, The Case Against Industrial Bio-Test Laboratories,” The Amicus Journal, 1983.
10. E. Marshall, “The Murky World of Toxicity Testing,” *Science*, vol. 220, pp. 1130 - 1132, 1983.
11. The New York Times, p. 34, 1983.
12. J. D. Cook, “Good Laboratory Practice versus CLIA,” [Online]. Available: https://www.westgard.com/guest16.htm.
13. K. Schneider, “IBT Labs’ trial reveals faked data,” In These Times, pp. 3 - 6.
14. P. von Stackelberg, “The Toxic Mist: The Use and Abuse of Pesticides,” Regina Leader-Post, 1980.
15. K. Schneider, “IBT - Guilty,” *Americus J*, 1983.
16. ICH, “History of ICH,” ICH, [Online]. Available: https://www.ich.org/page/history. [Accessed January 2022].
17. ICH, “ICH Q14: Analytical Procedure Development and Revision of Q2(R1) Analytical,” ICH, November 2018. [Online]. Available: https://database.ich.org/sites/default/files/Q2R2-Q14_EWG_Concept_Paper.pdf. [Accessed January 2022].
18. L. R. Snyder and J. W. Dolan, “Adjusting Conditions for a Routine Reversed-Phase HPLC Assay, Part I: Changing the Column,” *LCGC North Am.*, vol. 22, pp. 1146-1152, 2004.



References

19. J. W. Dolan and L. R. Snyder, "Adjusting Conditions for a Routine Reversed-Phase HPLC Assay, Part II: Changing Separation Conditions," *LCGC North America*, vol. 23, no. 2, pp. 118-127, 2005.
20. P. Petersson and M. Euerby, "An evaluation of the robustness of the Tanaka characterization protocol for reversed-phase liquid chromatography columns," *J. Sep. Sci.*, vol. 28, no. 16, pp. 2120 - 2129, 2005.
21. K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki and N. Tanaka, "Chromatographic characterization of silica C18 packing materials. Correlation between a preparation method and retention behavior of stationary," *J. Chromatogr. Sci.*, vol. 27, no. 12, pp. 721-728, 1989.
22. P. Zuvela, M. Skoczylas, J. Jay Liu, T. Baczek, R. Kaliszan, M. Wong and B. Buszewski, "Column characterization and selection systems in reversed-phase highperformance liquid chromatography," *Chem. Rev.*, vol. 119, no. 6, pp. 3674-3729, 2019.
23. T. Hanai, "In Silico Chromatography: Modeling a New Support for Alkyl-Bonded Phases and a Solvent Phase," *J. Anal. Bioanal. Tech.*, vol. 2, no. 2, pp. 111-117, 2017.
24. J. K. Field, M. R. Euerby and P. Petersson, "Investigation into reversed phase chromatography peptide separation systems part III: Establishing a column characterisation database," *J Chromatogr A*, vol. 1622, 2020.
25. "Welcome to the USP Column Equivalency Application," USP, [Online]. Available: https://apps.usp.org/app/USPNF/columns.html. [Accessed 2022].
26. L. R. Snyder, P. W. Carr and S. C. Rutan, "Solvatochromically based solvent-selectivity triangle," *J Chromatogr A*,. vol. 17, pp. 537-547, 1993.
27. USP, "General Chapter 467.pdf," USP, March 2007. [Online]. Available: https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/generalChapter467Current.pdf. [Accessed December 2021].