How Software Can Aid Decision-Making in Chromatographic Method Development

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Traditional trial and error experimentation is still often used in many analytical laboratories for method development, and while chromatographers will rely on their experience and intuition to select a starting point for method development, this approach is not reliably the most effective or efficient. Whereas in the case of simple samples the educated trial and error approach might be a reasonable strategy, it is increasingly less effective as the system becomes more complex.

The most difficult chromatographic method development scenarios are encountered in the development of stability indicating methods, or forced degradation studies in drug development. In these projects the chromatographer has to keep track of multiple impurities/degradants over many experimental conditions and chromatographic parameters. Tens, or even hundreds, of chromatograms can be generated in a fairly simple method screen.

The comprehensive approach to method development would be a thorough investigation of the design space for any given mixture or sample including buffer, column, solvent, time, temperature, etc. Given the time constraints and limited resources in any R&D laboratory, however, this type of broad scope investigation is unrealistic.

In order to provide a starting method within a reasonable timeframe, therefore, experienced separations laboratories will usually leverage corporate knowledge by employing a selection of well characterised standard methods as a starting point for new projects. These 'default' methods usually cover a large 'chromatographic space' including orthogonal columns, buffers, and solvent.

Even when past knowledge is used to limit the number of variables investigated in a new method development project, however, there is still a vast amount of data to process, analyse, review and make decisions on. It is risky to depend on a single human brain to deal with these large amounts of information. Furthermore, the question remains, does this approach help the analyst reach optimal design space for each sample, or would a more comprehensive investigation provide a better starting point?

The Holy Trinity of Effective Method Development

With the 'perfect storm' of technology at the chromatographer's disposal today, it is possible for multi-dimensional optimisation of parameters to be carried out without excessively burdening the chromatographer.

Efficient and thorough method development first requires a strategic approach. A combination of an understanding of the current project and the ability to apply organisational knowledge in context is important in helping to select a suitable starting point. Suitable instrumentation/hardware will enable a wide array of chromatographic conditions to be examined in parallel, and with minimal manual intervention (overnight, for example), if desired. Software is an ideal tool to streamline data collection, control, process, and interpret injections, and reduce transcription errors that might occur in manual review of large amounts of data. Finally, software can also help manage the knowledge gained in a project so that it can be re-applied in another iterative cycle for further refinement, or in future projects (as illustrated in Figure 1).

Context from Personal and Organisational Knowledge

Chromatographers apply their expertise and experience in every method development problem. In a perfect scenario, however, every project would start and finish at a knowledgebase where personal experience could be enhanced with organisational knowledge to provide a head-start.

Since chromatography is used throughout organisations across a wide variety of chemistry-related endeavours it is rare that a central repository of information is available. Results collected from different instruments are typically segmented and contribute little to organisational learning and intelligence. Chromatographic intelligence is typically scattered across a number of discrete silos including data files, Registry DBs, ELNs, lab notebooks, and reports. The multi-disciplinary nature of the information required at the beginning of a project (methods used in other parts of the organisation for the same/similar compounds; degradation patterns; salt screening data; solubility; stability; physicochemical properties—pKa, logP, logD) further contributes to the difficulty of one central system.

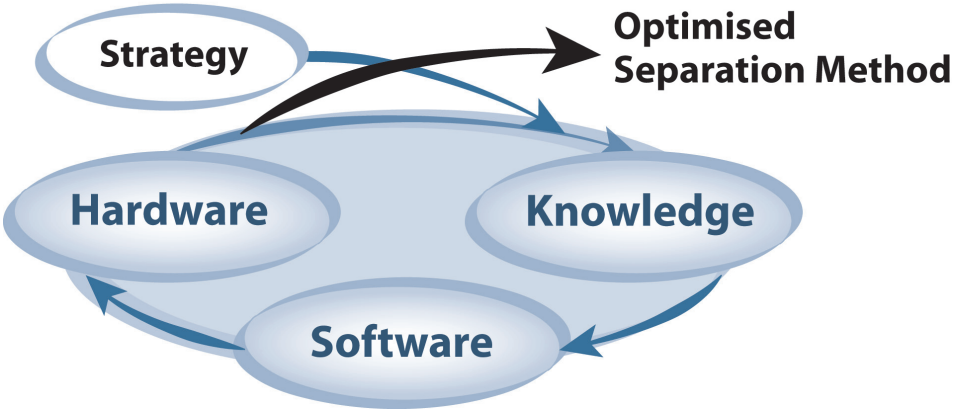

Figure 1: The holy trinity of effective method development—Knowledge, Software, and Hardware.

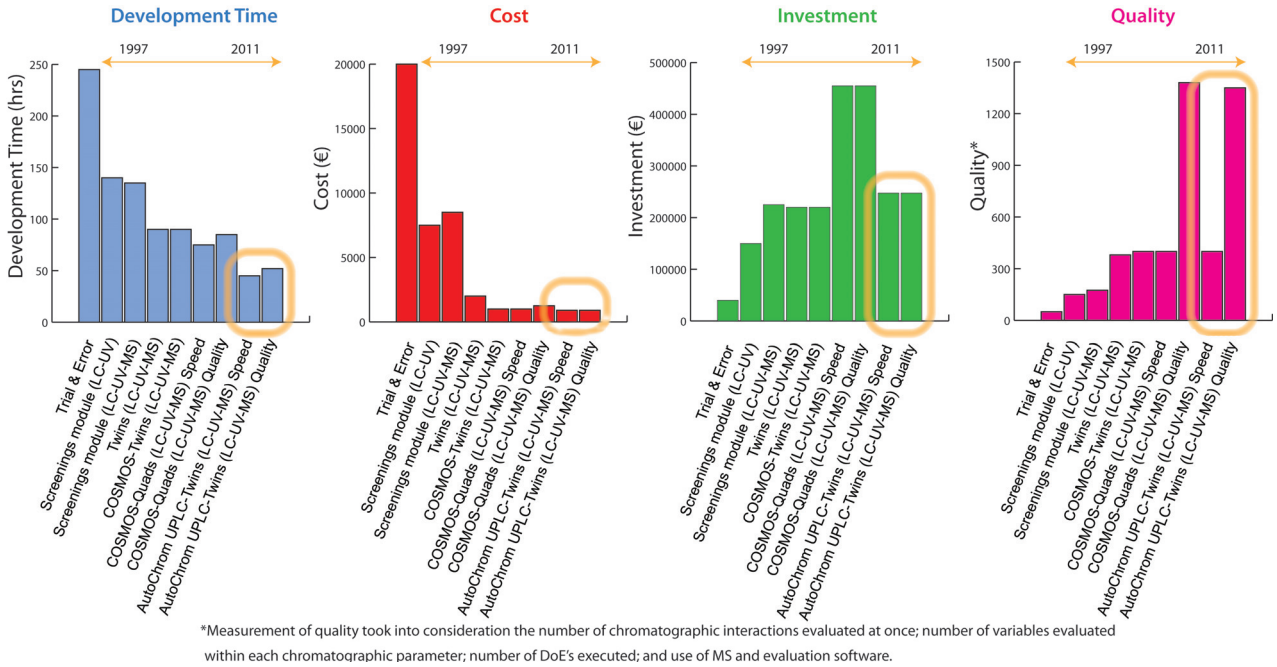

Figure 2: Progress of process output and return realised from refinement of method development—R. Sneyers (Janssen Pharmaceuticals Inc).

Hardware

Chromatography hardware has been developing at a rapid rate over the last decade and has seen a move to smaller column packing materials and much higher pump pressures, so-called 'Ultra High Performance Liquid Chromatography (UHPLC)' systems. The use of these higher efficiency systems, along with traditional HPLC instruments, coupled to multiple detectors (DAD/PDA, MS, ELSD, etc.) is a great aid to the method development scientist. These developments in hardware have greatly improved chromatographic performance and have potentially allowed for the easier tracking of components between injections.

Software

While instrument vendors provide software to run experiments and manage injections, there is little method development software available.

Ideally software for method selection and optimisation would treat the process in the same way as a chromatographer. It should process data so that this onerous task could be removed from the analyst, and track peaks across injections to facilitate faster decision-making. Built-in intelligence about the process would enable the software to make decisions based on specific requirements and criteria input by the chromatographer to achieve optimal design space. Optimisation should be an interactive process that allows the chromatographer to apply their experience and expertise to arrive at an acceptable method. Finally, the software should provide a single button click capability to save the knowledge gained from this process in a searchable database that could be accessed by colleagues to provide a head-start for future projects.

A chromatographic knowledgebase must not only include chromatographic data, but also its chemical context. This may include sample names, spectral peak assignments, or structures linked to elution data with methods. Furthermore, accumulation of the knowledge must require little manual effort on the part of the chromatographer and ideally be part of an approach to increase accuracy and efficiency.

Ideally at the conclusion of every method development all the knowledge gained from the project would be stored in a format that is accessible and searchable. Preferably this system would also integrate with a central knowledge management system to offer access to the wealth of intelligence gathered throughout the enterprise. Other than the ability to quiz a colleague face-to-face, digital information is ideal for preservation of intelligence and access to chromatographic knowledge.

Real-World Applications

Industry-leaders in chromatographic method development have adopted strategies that combine hardware, software, and knowledge, as a means to improve the productivity of their laboratories for higher return on investment.

Rudy Sneyers is a senior scientist in the small molecule method development department at Janssen Pharmaceuticals Inc (Belgium). For over a decade, he has made a concerted effort to improve the return on investment on (U)HPLC method development systems in their laboratories. Over the years his strategy for method development has evolved to one aligned with the holy trinity system, internally referred to as the 'AutoChrom UPLC-Twins [LC-UV-MS]' approach developed in the COSMOS project (Computer Organized Screening and Method Optimization System).

Through a combination of hardware improvements, the ability to learn from experimentation and re-use intelligence, and the appropriate application of software for organising, visualising, and tracking data, Sneyers' lab realised a significant reduction in development time, substantial reduction in FTE cost, and extensive improvements in the quality of methods (Figure 2 shows the progress of improvements).

Figure 2. Progress of process output and return realised from refinement of method development—R. Sneyers (Janssen Pharmaceuticals Inc).

The Janssen method development project is based on three principles:

- The use of scientific strategy (DoE)
- Introduction of a single quadrupole mass spectrometer alongside a PDA detector (LC-MS)
- Software designed for automated peak tracking and evaluation—ACD/AutoChrom MDS

ACD/AutoChrom MDS (Method Development Suite) is software developed by Advanced Chemistry Development, Inc (ACD/Labs). It was designed with the expert separations scientist in mind and closely tracks the workflow that chromatographers follow.

ACD/AutoChrom MDS enables the scientist to set up a method development strategy, whether that is a column/buffer/solvent screen followed by a gradient/temperature optimisation, or any combination of these parameters (and others). Once the method

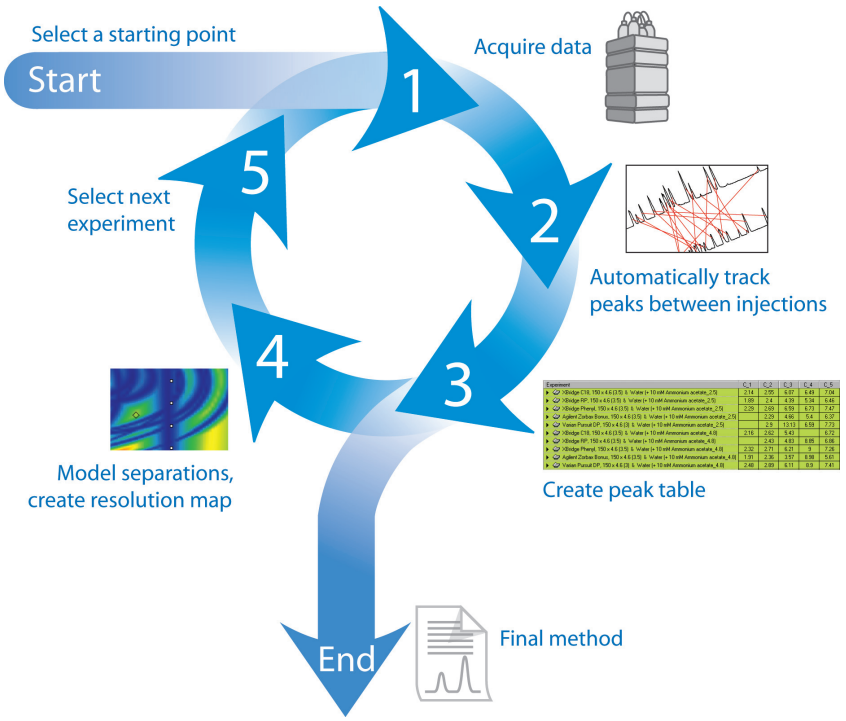

Figure 3: Automated workflow of QbD method development with ACD/AutoChrom MDS.

development strategy has been decided, the software interacts directly with the instrument (Agilent ChemStation or Waters® Empower™ software) to acquire the data and import it back into the project. Automated peak picking and peak tracking routines using DAD/PDA and MS spectra greatly reduce the data review time for the scientist.

"The cycle time for data analysis has been reduced from more than a week to within a day with ACD/Labs' method development software," agrees John Stafford (Eli Lilly, Indianapolis, USA) a long-term user of ACD/AutoChrom MDS.

Decision making tools within ACD/AutoChrom MDS allow the best experimental conditions from the Screening Wave to be quickly identified and moved to the Optimization Wave. Once experiments are acquired during Optimization, the software employs easy to use but powerful chromatographic modelling tools (ACD/LC Simulator) to interpret and resolve the system and determine the next best injection. This iterative approach to method development, between scientist and software, is not only intuitive to the chromatographer but also results in a vast reduction in the number of injections required to solve the task.

Tom van Wijk who works in the method development and validation department at Abbott Healthcare Products BV (Netherlands) also uses a combination of information, hardware, and software for effective method development and has found that "the development of a high quality stability indicating method within two weeks is feasible" when using this system. Van Wijk's lab employs a variety of software tools to aid method development. Among these is ACD/Labs' physicochemical property prediction software used to estimate properties for known and potential impurities (pKa, logP, logD) to provide 'context'. They also use ACD/AutoChrom MDS, which allows them to automate HPLC method screening. In order to leverage legacy knowledge a database is maintained to help select starting points for new projects.

ACD/Labs uniquely provides a multi-vendor, multi-technique knowledge management platform (ACD/Spectrus) to help homogenise the knowledge gathered daily by scientists along with its chemical context and human interpretation. A number of knowledge management solutions are provided for separations laboratories that can help with column selection, chiral or achiral method selection, and impurity tracking. The comprehensive AutoChrom product will save all the data from initial screening procedures through to labelling individual chromatographic peaks of the optimised method. With the single click of a button decisions made in the project are preserved in a homogenous environment that offers access to the wealth of knowledge. This collaborative Spectrus environment offers the chromatographer a central repository from which to gather the information produced by disparate labs at the beginning of a method development project. The data may be searched by a variety of structural, chromatographic, spectroscopic, and textual parameters.

"Reliability, robustness, and lifetimes of methods have improved, and at the same time we have reduced the loss of interpretation information and expensive retesting of samples…the QbD approach to method development aided by ACD/AutoChrom MDS (important for method quality) will provide return on investment within a short time," Rudy Sneyers (Janssen Pharmaceuticals Inc.).

Conclusion

The most effective strategy for method development should be a combination of hardware, software, and application of personal and organisational knowledge. The demand for greater efficiency and productivity in all chemical R&D enterprises can only be met by using all the available tools.

Chromatography software tools in the separations laboratory make it possible to distil data in a highly automated fashion and aid data analysis and visualisation. This helps the analyst to arrive at decisions faster and with greater confidence. Software systems designed for creation of chromatographic knowledgebases and preservation of intelligence, as an extension of everyday scientific activities, help to streamline chromatographic method development, method selection, and data interpretation.

While there are not many vendors of method development software, the benefit of including such software is a reduction in the number of injections/experiments required for new samples and ultimately, greater efficiency. Furthermore, organisations that are able to provide a central resource of unified laboratory intelligence to their scientists will benefit from the re-use of legacy knowledge with improved productivity and better allocation of resources.

Acknowledgements

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New Headspace Sampler for GC and GCMS

Shimadzu has launched the HS-20 gas chromatography headspace sampler for accurate analysis of an even wider range of volatile com-pounds with boiling points ranging from low to high. The HS-20 heats liquid or solid samples sealed in a container to a specific temperature, and injects the volatile compounds diffusing into the gaseous phase into a GC or a GCMS. These systems are widely used in the fields of environmental and pharmaceutical applications as well as in materials and food products analysis and forensics. The HS-20 will support the-se analyses particularly in testing and inspection organizations.

The unique configuration of flow lines and the oven enables the anal-ysis of high boiling point compounds while minimizing carryover. Using the electronic cooling trap, it is also possible to concentrate the headspace gas for analysis of compounds with extremely high sensi-tivity.

Headspace samplers enable easy analysis of volatile compounds. They are used in various fields requiring higher reliability, such as analysis of VOCs (volatile organic compounds) in the environmental and quality control applications of pharmaceuticals whereas in food products and materials control a wide range of volatile compounds with low to high boiling points has to be detected with high sensitivity in order to provide accurate qualitative and quantitative results for numerous measurement targets.

For more information www.shimadzu.eu

New HILIC Column for UHPLC

Nacalai Tesque (Japan) introduces a new HILIC column for UHPLC. COSMOSIL 2.5HILIC, with 2.5 micron particles, provides superior separation and significantly lower back pressure than sub 2 micron C18 stationary phases. Furthermore, this novel triazole bonded silica stationary phase provides higher polarity than non-modified silica columns, resulting in stronger hydrophilic interactions. The positively charged triazole stationary phase also offers an anion-exchange mechanism, enabling the strong retention of acidic compounds.

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