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Challenges and Considerations for Building an Automated Method Development System

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INTRODUCTION

Automated chromatographic method development systems have been offered for a number of years, with varying degrees of effectiveness. Difficulties in tracking peaks between experiments can limit these systems, especially for samples such as impurities and degradants which may have many trace components to resolve.

Automated method development systems typically lack versatility, meaning that established method development approaches must be adjusted in order to fit the capabilities of the automated system. Finally, for complex problems, method development may involve many experiments with many samples (including composite samples), and a large amount of data can be generated. It can take a significant amount of human time and effort to track, review, and manage this data.

A new system for automated method development, ACD/AutoChrom, is currently under development, and addresses some of the weaknesses of earlier configurations. AutoChrom uses both UV-Visible and MS detection to unequivocally track and resolve trace components, performing a chemometric evaluation of these detection techniques. The system also includes data handling and storage systems designed to quickly summarize the information, and reduce the time required for data review and report creation. A wide array of instrument configurations and method development approaches are supported, including column and mobile phase screening, making the system flexible for many types of laboratories.

SYSTEM FLEXIBILITY

In order for software to be adopted within a laboratory, it must support common method development workflows. The software should adapt to the chromatographers' current workflow, not the other way around. This has been a major limitation of previously reported automated method development systems. AutoChrom supports a number of approaches to method development. The supported method development workflows are described elsewhere.[1]

COLUMN AND MOBILE PHASE SCREENING

One of the most common ways to start a method development project is to screen multiple stationary and mobile phases. The best system is then selected for further method optimization. Because screening experiments are so common, automated method development software should include a module to execute and, more importantly, evaluate the results of column, mobile phase, and/or buffer screening experiments.

One of the major challenges with evaluating the results of screening experiments is tracking peaks from run to run. Peak shapes and retention order can change substantially from run to run, making it extremely difficult to reconcile data. In order to effectively evaluate the results of screening experiments, the ideal automated method development system should be able to automatically and reliably track peaks from run to run.

Column screening is especially important during the development of stability-indicating methods, where the number and identity of analytes are unknown. It is important to analyze the key sample set using orthogonal methods to be sure that no components are overlooked due to coelution.

DATA PROCESSING

Peak Tracking Between Runs

Whether developing methods manually, or by using a software optimization system, chromatographic peak matching between runs is critical. All peaks must be tracked across all experiments. In software-assisted method development, the peaks must be matched before the results can be passed to method optimization and modeling software (e.g., DryLab, ACD/LC Simulator).

Humans use spectra, intuition, and test injections to match peaks across runs, but this can be a very time-consuming process, particularly when dealing with complicated samples; computers are more limited. A particular challenge (for both humans and computers) is the detection and tracking of low-level peaks arising from impurities or degradants.

LC/UV Data

The most common hyphenated HPLC detector is the LC/UV, or diode array detector (DAD). This detector has the advantages of being inexpensive and easy to use. Disadvantages of LC/UV detectors are its relatively poor detection limits, lack of specificity (similar compounds yield similar spectra), and, most importantly, the fact that an analyte spectrum can change as mobile phase conditions are altered. The latter is particularly limiting for buffer and mobile phase screening.

Despite the limitations of UV-detection, LC/UV peak tracking still yields useful information,[2] particularly when used to complement LC/MS data from the same experiment. AutoChrom includes UV-MAP (UV-Mutual Automated Peak Matching), an algorithm designed for the automatic matching of two or more LC/UV data sets.[3] The first step in UV-MAP is baseline correction, which is performed with an ACD/Labs' proprietary algorithm. After baseline correction is performed, peaks are picked, and a pure component spectrum is generated for each. Peaks are matched using UV spectral similarity, combined with peak areas.

LC/MS Data

In recent years, the application of LC/MS to chromatographic method development has become more common. MS detectors provide good sensitivity, analyte specificity, and detection limits. Matrix effects can suppress (or enhance) ion signals, but if the presence of ions is used rather than relative intensities, the information is fairly insensitive to the solvent environment. However, when working with LC/MS, the choice of mobile phases and buffers is limited, so it is not yet a routine tool in all laboratories.

MS peak tracking is achieved with the MS-MAP algorithm. MS-MAP uses the presence of ions in a mass spectrum to match peaks across runs. Mass spectrum interpretation, including the assignment of [M+H]+, adduct ions, and possible fragment ions, provides additional clues for peak matching. This interpretation is based on ACD/IntelliXtract, which is powerful software for component extraction and spectrum interpretation for full-scan LC/MS data.

Because mass spectral data is discrete, rather than continuous (like UV spectra), we assume that ions associated with a particular peak described in a given run will be represented in all other runs. When the peak is not resolved, this spectrum will be combined with another, but the signals may still be resolved. This is illustrated in Figure 1. MS-MAP will still be able to track this peak throughout all runs; the only requirement for detection of a given mass by the MSMAP algorithm is its presence as an isolated component in at least one run.

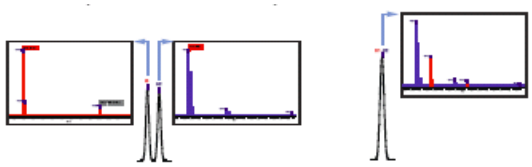

Figure 1: Coeluting components may be deconvoluted and tracked by MS-MAP if the analyte is isolated in at least one run. If ions from two separate components are detected together, two separate components with the same retention time are recorded in the Peak Table.

Reconciling Detectors and Peak Deconvolution

MS and UV detection yields complementary information, so it makes sense to use both detectors when possible. Analytes that do not ionize will not be visible with mass spectrometry, but may have a chromaphore such that they are visible to UV. Conversely, analytes without a chromaphore, or analytes below LC/UV detection limits, may still be detected by mass spectrometry. Additionally, while some analytes will have very similar UV traces due to similar functional groups, molecular weights and fragment ions may be different, such that MS can differentiate compounds where UV cannot.

AutoChrom provides a method for reconciling UV and MS detectors for a single run based on simple retention time shifts. Additionally, viewing all of the

elution data acquired over the course of a method development project provides additional information to distinguish coeluting peaks.[4-5] AutoChrom can correlate all LC/UV and LC/MS detector traces across all experiments using the proprietary NDMC algorithm. If a given UV-detected peak coelutes with a given MS-detected peak across all sets of conditions, then the component is deemed a single analyte. If the MS and UV peaks diverge appreciably across (one or more) injections, the signals coelute. In essence, a multi-dimensional retention time index is used to reconcile detectors, and deconvolute coeluting peaks. When both MS and UV detection is used, it is possible to detect an analyte even if its pure component spectrum is not available (i.e., it is always coeluting with another analyte, but not with the same analyte in every injection). This is illustrated for a three-component system across two injections in Figure 2.

The final results of MS and UV peak matching are summarized in a peak table, which is then passed to LC Simulator (part of AutoChrom) for evaluation of screening results, or method optimization. The method optimization cycle used by AutoChrom is described elsewhere.[6]

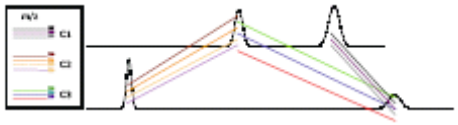

Figure 2: Component 3 is not resolved in either separation, but can still be and resolved by AutoChrom.

Applicability of MS-MAP and UV-MAP

The complexity of samples, S/N ratios, and resolution of components are all factors which affect the performance of MS-MAP and UV-MAP. The algorithms work best with small solvent condition changes, and perform reasonably well in the case of solvent strength (%B) and temperature optimization. Solvent and pH screening can mean large changes in solvent conditions, which limit the applicability of spectral-based peak tracking. MS-MAP is ideal for column screening experiments, with accuracy approaching 100%. All other screening and optimization experiments may require some manual data review.

EVALUATING THE RESULTS OF SCREENING EXPERIMENTS

Automated peak matching greatly assists with data processing for screening experiments. The next step is to choose the best system for further optimization (e.g., gradient, temperature, and/or solvent strength optimization).

When none of the systems can achieve acceptable resolution of all peaks, it can be difficult to determine which systems are the best for further method development.

AutoChrom provides a way to rank the suitability of a system for further method development. Screening results are ranked according to the average resolution, peak equidistance, and/or equiresolution. AutoChrom can then automatically select the best method(s) for further optimization. Alternately, the software can wait for the chromatographer to manually select the best results, while providing a convenient interface for data review. Figure 3 shows the results of column screening experiments.

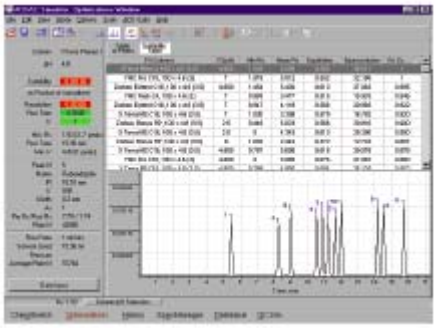

Figure 3: Results of column screening experiments are summarized in a table, and can be ranked by minimum resolution, equiresolution, or other parameters so that the optimal method for further optimization can be easily selected.

DATA MANAGEMENT

The use of MS and UV detection in tandem provides benefits for peak tracking and analyte detection. However, when two or more detectors are used for each injection, this yields a lot of individual data files. Replicate injections may be performed, and standards and blanks may be analyzed in addition to the sample(s). The amount of data collected over the course of even a straightforward project can quickly become overwhelming.

Data management therefore is a critical piece of an automated method development system. Results should be clearly communicated to the chromatographer. AutoChrom provides a project management interface that summarizes experiments, analyte retention times, chromatograms, spectra, chemical structures, and other information. Links to

raw data files are maintained, so that data may be conveniently accessed for review and reprocessing, if necessary. The details are described elsewhere.[7]

Figure 4: Data is summarized in a project management interface. Retention times for each method, sample, replicate injection, and detector are summarized in a table. The original data files are hyperlinked, so that data can be reviewed and reprocessed if necessary.

CONCLUSIONS

Automated method development systems should support screening experiments, as this is one of the most common ways to start method development. The major challenge lies in tracking peaks between experiments, as peak retention times and shapes can change substantially with changes in stationary and mobile phase composition.

Automated peak tracking makes automated screening experiments feasible. Peaks may be tracked using UV or MS detection. Automated MS-based peak tracking tends to be more accurate than UV peak tracking, which is limited by detector characteristics. When both detectors are used in tandem, peaks may be reconciled across detectors using a multi-retention time index, allowing coeluting components to be deconvoluted.

Finally, the use of multiple detectors results in multiple individual data files for a single injection. Over the course of method development, tens to hundreds of injections may be performed, which means that data management is a crucial part of any automated method development software. The software interface should clearly communicate the results to the chromatographer.

REFERENCES

1. McBrien, M., M. Bayliss, A. Galin, E. Kolovanov, V. Lashin. PITTCON 2006, Orlando, FL, USA. March 12–17, 2006.
   http://www.acdlabs.com/download/publ/2006/pittcon_autochrom.pdf
2. Swartz, M.E., Brown, P.R., Chirality, 8 (1996) 67–76.
3. Bogomolov, A., M. McBrien, Anal. Chim. Acta, 490 (2003) 41–58.
4. McBrien, M., E. Kolovanov, V. Tashlitsky, A. Vazhentsev, M. Bayliss, V. Lashin. 29th International Symposium on High Performance Liquid Phase Separations & Related Techniques (HPLC) 2006, San Francisco, CA, USA. June 17–23, 2006.
   http://www.acdlabs.com/download/publ/2006/hplc06_ndmc.pdf
5. McBrien, M., M. Bayliss, A. Galin, E. Kolovanov, V. Lashin. PITTCON 2006, Orlando, FL, USA. March 12–17, 2006.
   http://www.acdlabs.com/download/publ/2006/pittcon_map.pdf
6. Antler, M. 29th International Symposium on High Performance Liquid Phase Separations & Related Techniques (HPLC) 2006, San Francisco, CA, USA. June 17–23, 2006.
   http://www.acdlabs.com/publish/publ06/hplc06_md.html
7. Janssens, W., A. Pluym, R. Sneyers. PITTCON 2006, Orlando, FL, USA. March 12–17, 2006.
   http://www.acdlabs.com/download/publ/2006/pittcon06_lc-ms-dad.pdf