

Systematic Development of Stability-Indicating Methods

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This article discusses some of the benefits and challenges associated with maintaining individual degradation samples for LC-MS-UV method development. The latest advancements for automated data interpretation, including automated LC-MS and LC-UV peak tracking and detector reconciliation, are described. These advancements allow a virtual composite sample to be created post data acquisition and transferred to LC optimization software for systematic, efficient method development.

Chromatographic method development can be a challenging process. There is a practically unlimited number of chromatographic conditions that may be considered in designing an effective technique for quantifying impurities in a given sample. A hit-or-miss approach can be used when chromatographic samples are very simple or when analysis will not be performed enough times to reward excellent method design. A more rigorous, scientific approach is appropriate for complex problems or for design of robust methods that will be applied thousands of times.

Modern pharmaceutical practices require quantification of impurities at levels of 0.05–0.1% of the active compound. This



requirement can be particularly challenging when addressing stability samples. Because method development must occur without standards available for analysis, a typical approach is to generate the expected degradation products through a series of forced-degradation experiments on the active compound, and ideally design a method for the analysis of all resulting degradants with a single chromatographic method. The development of this method can be a daunting task when one considers that more than 20 impurities may be observed, pushing the limit of the resolving power of HPLC.

Computer-Assisted Chromatographic Method Development

The major principles of systematic, computer-assisted chromatographic method development are well-established.¹ With enough experiments, the influence of practically any

continuous variable on component elution can be modelled with one of several commercially-available software packages.²⁻⁴

The most common approach to optimization is the modelling of the ratio of organic solvent to water in reversed-phase HPLC, which can be described by a linear prediction equation relating the capacity factor for each component, k' , to solvent strength. On this basis, two experiments (or, for more accuracy, three) can be used to build a prediction model, and predict the retention times of all possible gradient and isocratic programs. Results are typically visualized with maps showing the resolution between the two closest eluting components, giving an overview of the potential experiments, which allows the chromatographer to quickly determine the best experiment to try next. Temperature, buffer concentration and other parameters may also be modelled in a similar fashion, albeit in



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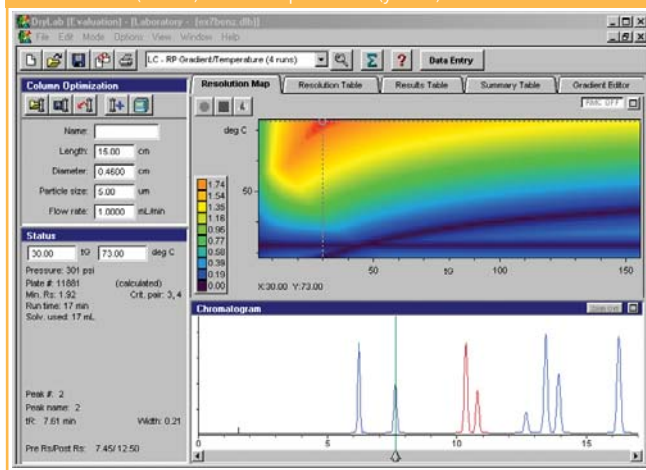
most instances using more complex retention models. Using these same principles, two or more variables can be modelled at the same time. This is illustrated in Figure 1.

Perhaps the biggest challenge associated with systematic method development is the requirement that all components be tracked across all experiments to produce a retention time model. The more complex the modelling system, the more experiments must be conducted, and furthermore, the more complex the sample, the more complex the peak extraction from the elution data. For example, a sample with eight components such as the one illustrated in Figure 1 may require 16 injections to optimize the conditions for a non-linear, two-dimensional (2D) model (e.g., optimizing the temperature and per cent organic). If 8 components are monitored through 16 runs, this means that there are 128 peaks that must be identified and matched to build the prediction model.

Composite Samples for Forced Degradation Studies

The compounds that result from the degradation of a given pharmaceutical can be generated in a relatively short period of time through the use of forced-degradation experiments in which various agents are used to stress the sample. Conditions commonly used will be acid, base, heat, UV-light and peroxide. In theory, any impurity that will be observed based on the degradation of a given sample over extended periods of time

Figure 1: Resolution map (top) and simulated chromatogram (bottom) created with method optimization software.² The colours in this resolution map describe the resolution of the critical pair (the two closest-eluting peaks) while varying % acetonitrile (x-axis) and temperature (y-axis).

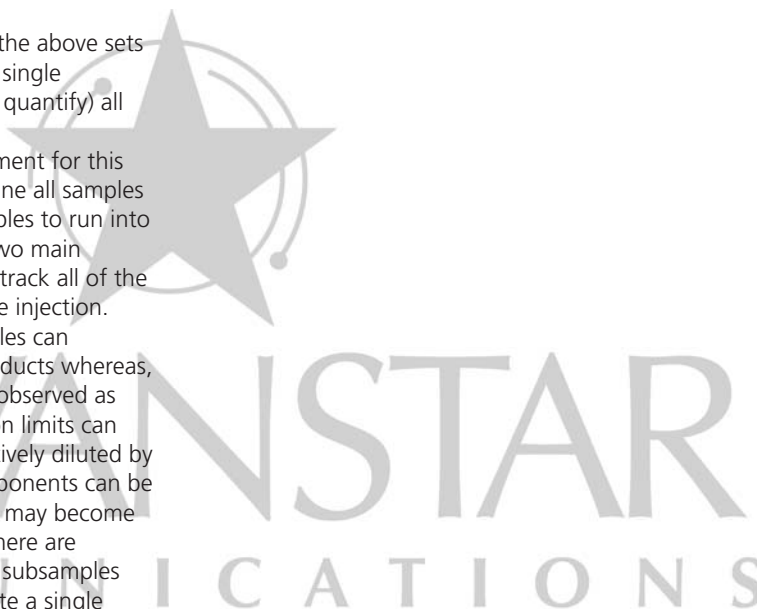


can be generated quickly using one or more of the above sets of conditions. The ideal scenario is to develop a single chromatographic method to separate (and thus quantify) all relevant impurities and degradants. When the chromatographer commences method development for this kind of sample, the temptation can be to combine all samples post-degradation to reduce the number of samples to run into a single injection. However, this approach has two main drawbacks. The first is that it can be difficult to track all of the components when they are contained in a single injection. Co-eluting components from separate subsamples can potentially be attributed to fragmentation or adducts whereas, if they were injected individually, they could be observed as unique components. More importantly, detection limits can become an issue when each subsample is effectively diluted by a factor of as much as five times. Whereas components can be detected relatively easily at the 0.1% level, they may become untraceable at the 0.02% level. It is clear that there are significant benefits to maintaining independent subsamples and combining the results post-injection to create a single composite sample.

The composite sample approach has been resisted by some researchers because of the fact that even the most efficient method development requires the examination of a significant number of sets of chromatographic conditions. Using five degradation samples plus a blank introduces a six-fold increase in the number of experiments required. However, instrument time is inexpensive in real terms, as autosamplers allow samples to be analysed overnight. In addition, the new availability of UPLC provides the opportunity to work with multiple degradation samples in a given workflow without a substantial increase in instrument time beyond that of "monotone" samples with traditional HPLC.

Peak Tracking Across Samples and Injections

The much larger number of injections required for method development using composite samples creates a problem — extracting and tracking elution data for all components across all subsamples and all conditions, as illustrated in Figure 2. Peak tracking must be performed whether using a software-assisted or manual approach to method development. A degradant may form via multiple degradation pathways under different degradation conditions, so it is necessary to track peaks across samples if maintaining multiple degradation samples. In many instances, peak matching is performed manually. When using multiple samples, it can take hours or even days to manually reconcile all of the data from a forced-degradation study.



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Hyphenated detection techniques such as LC-UV (photodiode array detector) or LC-MS provide at least a partial solution to this problem. Chemometric tools have been developed for the tracking of multiple components across chromatographic conditions for LC-UV data sets⁵⁻⁷ and for LC-MS datasets.⁷ These utilities can speed the detection of components a great deal by creating a list of components and extracting elution data for each under each set of conditions. Components are then matched based on spectral similarity. Matching peaks by UV spectral similarity has two main drawbacks. The first is that the UV spectra of two related components can be very similar, which can make it difficult to unambiguously match peaks across injections. Second, a variation particularly in pH between methods may significantly alter the spectrum for a component, which can make it impossible to track changes by spectral similarity.⁶

Peak matching with LC-UV detection may not be selective enough to distinguish between similar components, but MS detection gives additional information, such as the component mass, as well as fragment, isotope and adduct information. This provides additional confidence in the peak matching. There are a number of limitations for MS-based peak tracking, including noise and ionization-related issues when buffers are changed. Automatic peak tracking for LC-MS has been reported, and was found to significantly outperform LC-UV peak tracking.⁷ In this approach, software extracts the pure-component mass spectrum for each component by aligning the individual ion chromatogram peaks. The pure component mass spectrum for each component is then interpreted — the protonated molecule $[M+H]^+$, adduct, multimer and fragment ions are assigned. Chromatographic

peak matching is performed using the pure component mass spectrum. LC-MS peak tracking requires that components ionize under the conditions used, but has excellent success beyond this limitation. The logical conclusion is to use UV and MS detection in tandem and then reconcile components between detectors. Automating LC-UV or LC-MS peak tracking can reduce the manual data interpretation burden on the analyst, making method development using multiple degradation samples more feasible.

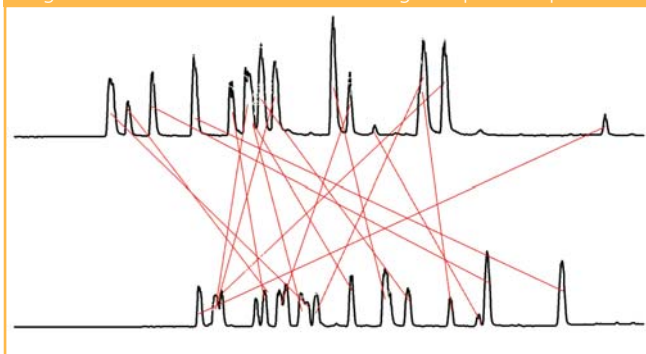
LC-UV and LC-MS Detector Reconciliation

One of the major benefits of using both LC-UV and LC-MS detection is the complementary nature of the systems. Components that do not ionize will not be visible with MS, but may have a chromophore such that they are visible to UV. Conversely, compounds without a chromophore, or components below LC-UV detection limits, may still ionize and show up in the LC-MS trace. Additionally, while functional groups may be the same, such that components have very similar UV traces, molecular formulae may be different, such that MS can differentiate compounds where UV cannot. This orthogonality presents another challenge; how does a chromatographer know if two traces with different detectors are the same component showing on both detectors or two co-eluted components? In the instance of individual injections, the chromatographer is forced to conclude that signals with the same retention times under a given set of conditions are the same component.

Viewing all of the elution data acquired over the course of a method development project can provide an additional clue. Because a given peak will be tracked across all experiments for both detectors, the detector traces can be correlated across all experiments simultaneously.⁸ In essence, a multidimensional retention time may be used to reconcile detectors. If a given UV-detected component co-elutes with a given MS-detected component across all sets of conditions, then the component is deemed to be the same. If the components diverge appreciably, then the components are deemed to be unique. Additionally, a component that co-elutes with two different components across two different methods may be resolved using the MS and UV spectra. This is illustrated for a three-component system across two injections in Figure 3.

The result of the combination of chemometric peak tracking with detector reconciliation is fast, effective reduction of a huge amount of LC-MS/UV data to a list of components with their elution data. The elution data for each degradation condition may be combined post-acquisition to

Figure 2: Peak matching from run-to-run can be complicated for samples with many components because of co-elution, large shifts in retention times and changes in peak shapes.



create a "composite chromatogram", as shown in Figure 4. The composite chromatogram summarizes signals for all detectors and subsamples into one overall set of elution data for consideration in experimental modelling. This information can be transferred to method optimization software for systematic method development.

Conclusion

Maintaining individual degradation samples for a method development project provides better detection limits and fewer components to resolve per sample, but poses a major challenge for data handling and interpretation. A large number of injections and detector traces as well as subsamples must be compiled, and challenges lie in peak tracking across samples, experiments and detectors. Recent advancements in software allow automated peak tracking for LC-UV and LC-MS, and detector reconciliation. The individual degradation samples may be combined post-analysis to create a virtual composite sample, which may then be transferred to method optimization software for systematic method development.



Figure 3: An illustration of the value of multiple detector reconciliation across all experiments. Component B is unresolved chromatographically in either of the experiments shown. The MS signal for B could potentially be attributed to fragmentation of component A in the first experiment, and fragmentation of component C in the second experiment. However, the signal for Component B in the LC-UV trace in most cases can resolve this issue. Similarly, MS detection can address ambiguities for components with similar UV spectra.

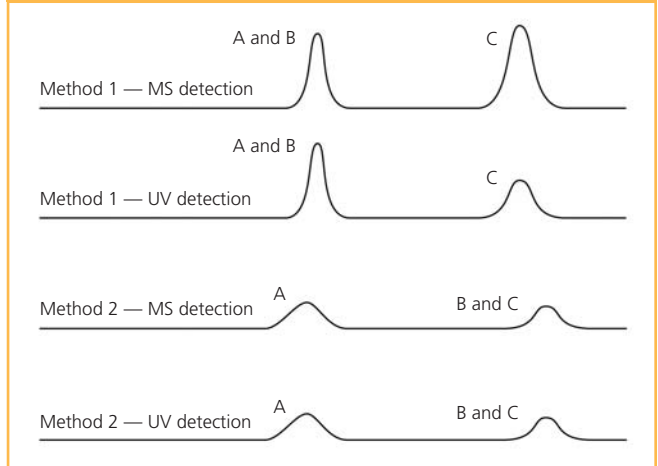
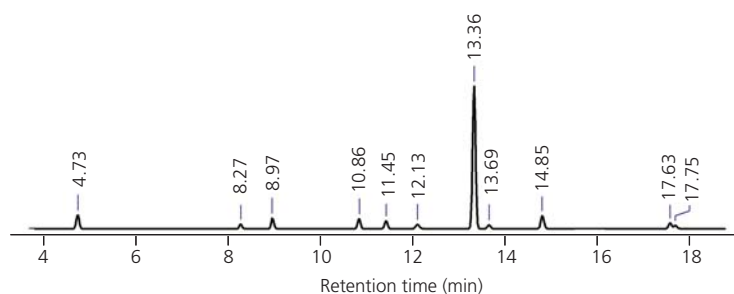


Figure 4: A composite chromatogram for two subsamples with LC-UV and LC-MS detection with summary peak table. The chromatogram(s) for each subsample are combined post-acquisition to create a virtual composite sample.



Average retention times for composite sample	8.97	8.27	11.64	12.13	10.86	13.81	13.36	14.85	17.63	17.75	4.73
Subsample 1 — acid degradation	9.02	8.37	11.64	12.32	10.78	-	13.35	14.44	17.56	17.91	4.91
S1-UV	9.02	8.37	11.63	12.31	10.78	-	13.35	14.44	17.56	17.9	-
S1-MS	9.02	8.37	11.65	12.32	10.78	-	13.35	14.44	17.56	17.91	4.91
Subsample 2 — heat degradation	8.92	8.18	-	11.94	10.94	13.81	13.37	15.25	17.71	17.59	4.55
S2-MS	8.92	8.18	-	11.94	10.94	13.81	13.37	15.25	17.71	17.59	4.55
S2-UV	All components below limit of detection.										

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References

1. L. R. Snyder, J. J. Kirkland and J. L. Glajch, *Practical HPLC Method Development*, 2nd Edition, Wiley-Interscience (1997).
2. DryLab, Rheodyne LLC, <http://www.rheodyne.com/products/chromatography/drylab/>, Accessed 23 November 2006.
3. ACD/Method Development Suite, Advanced Chemistry Development, Inc., www.acdlabs.com/meth_develop/, Accessed 23 November 2006.
4. ChromSword, Iris Technologies International, Ltd., <http://www.iris technologies.net/ChromSword/ChromSwordAutoMain.htm>, Accessed November 23, 2006.
5. M.E. Swartz and P.R. Brown, *Chirality*, **8**, 67–76 (1996).
6. A. Bogomolov and M. McBrien, *Anal. Chim. Acta*, **490**, 41–58 (2003).
7. G. Xue et al., *J. Chrom. A*, **1050**, 159–71 (2004).
8. M. McBrien, 29th International Symposium on High Performance Liquid Phase Separations & Related Techniques (HPLC) 2006, San Francisco, California, USA. 17–23 June 2006. http://www.acdlabs.com/publish/publ06/hplc06_md.html

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