Waters

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Benefits of Automated HPLC Method Development and Transfer in Conjunction with Fast Chromatography

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Abstract

The Waters Automated Method Development System (AMDS) provides an efficient unattended solution to method development and transfer. We will discusses a simplistic approach to develop and transfer methods to newer technology effortlessly while at the same time reducing cost and increasing profitability and lab efficiency.

There is an enormous demand on today's chemist to increase method development productivity but at the same time reduce costs. However, these two expectations are greatly hindered without today's growing technological advances. Manual HPLC is labor intensive, time consuming and often imprecise hence the need for an automated process. As well, many chemists are not taking advantage of newer column chemistries because of their lack of experience with them. To date, there has been no efficient approach to reap the benefits that can ultimately save analysis time and money. Waters Automated Method Development System (AMDS) provides efficient routes to achieving these demanding tasks while providing utilization of the advantageous technologies in the field of column chemistry and instrumental advances that can present noticeable cost savings.

Objective

Updating Chemistry for QC reasons:

Diltiazem

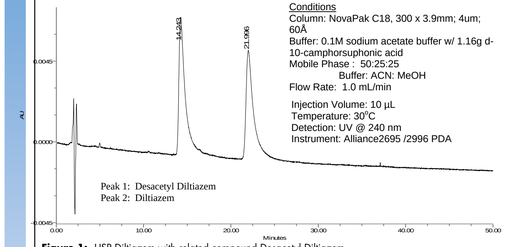
- Update the present USP method with newer column chemistry using AMDS
- Explore Intelligent Speed (*IS*™) columns
- Optimize assay for validation
- Establish cost of analysis and possible ramifications of improvements.

Investigating Newer Instrumentation for R&D reasons:

Ginger Root Assay

- Investigate and optimize new chemistry with HPLC
- Simulate ACQUITY UPLC™ parameters using AMDS data
- Prove accuracy

USP Diltiazem and related compound



Conditions
Column: NovaPak C18, 300 x 3.9mm; 4um; 60Å
Buffer: 0.1M sodium acetate buffer w/ 1.16g d-10-camphorsuphonic acid
Mobile Phase : 50:25:25
Buffer: ACN: MeOH
Flow Rate: 1.0 mL/min

Injection Volume: 10 µL
Temperature: 30°C
Detection: UV @ 240 nm
Instrument: Alliance2695 /2996 PDA

Figure 1: USP Diltiazem with related compound Desacetyl Diltiazem.

Other examples may use different columns such as µbondapak, but the common thread within many QC labs is that their SOP methods may be largely based on old validated methods using older column chemistry technology. Even though the tailing of this chromatogram is less than desired, the USP parameters are met: Resolution is greater than 3.0 and the theoretical plates are greater than 1200. Even though, these tailing issues may have reproducibility issues which can possibly create downtime in a QC lab trying to achieve suitability or even faulty time consuming OOS results.

AMDS Set-up

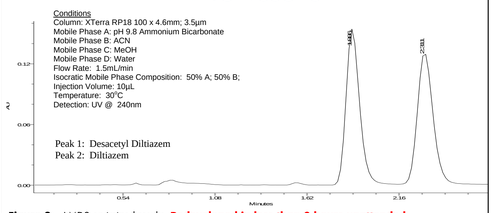
Analytical Goals:

Priority: Run time more important than resolution
Resolution: > 3.0 (priority may allow for small compromise for info. purposes)
Run time: < 5.0 minutes
Separation: Isocratic preferred
Max. Pressure: ~3000 psi
Min. t0: 2 times t0 (typical for USP methods)

AMDS Recommended Starting Conditions:

pH 9 (or higher) Ammonium Buffer
XTerra MS C18 and XTerra RP18 columns (100mm x 4.6mm; 3.5µm)
USP states 240nm (but MAXplot 210-400nm may have been used)

AMDS Results



Conditions
Column: XTerra RP18 100 x 4.6mm; 3.5µm
Mobile Phase A: pH 9.8 Ammonium Bicarbonate
Mobile Phase B: ACN
Mobile Phase C: MeOH
Mobile Phase D: Water
Flow Rate: 1.5mL/min
Isocratic Mobile Phase Composition: 50% A; 50% B;
Injection Volume: 10µL
Temperature: 30°C
Detection: UV @ 240nm

Figure 2: AMDS optimized results **Redeveloped in less than 3 hours unattended.**
A standard AMDS set-up found a separation in less than 3.0 minutes. Peak shape was improved while still maintaining all USP requirements. With the higher pH and newer column technology, peak shapes have sharpened while allowing to work in a more pH robust zone for these basic components (as described per Figure 3). Actual results yielded a resolution of 3.2 with over 4000 theoretical plates meeting USP requirements.

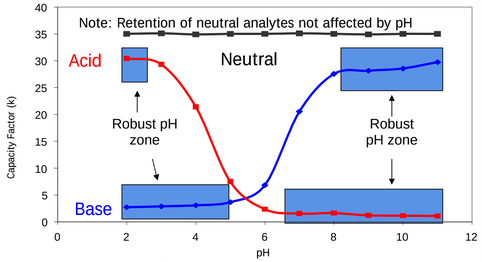


Figure 3: RP retention behavior of Neutral, Acidic, and Basic Compounds
This chart illustrates how working at the proper pH can allow for more robust methods.

Taking that extra step yields profits

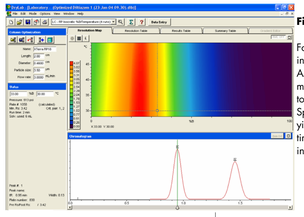
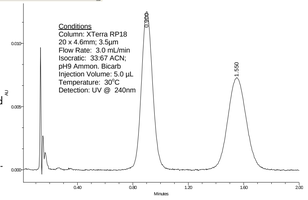


Figure 4: Drylab Resolution Plot

For further post processing, using the resulting Drylab resolution plot obtained from the AMDS result the user can manipulate numerous simulations of various chromatographic conditions. *IS*™ (Intelligent Speed) column parameters were entered to yield predictions that could decrease run time while maintaining resolution and yielding a higher throughput potential.

Figure 5: Actual injection of Diltiazem method to verify Drylab predictions.

With this 2 component USP drug mixture, *IS*™ columns are a good choice to speed analysis time which could eventually save millions of dollars for a QC lab. In the end this improved optimized method could decrease solvents used, decrease trouble-shooting due to OOS or suitability issues, decrease batch release times, decrease inventory and inevitably increase profitability. Well worth the week of validation.



Keeping Up with Instrument Technology

Redevelopment and validation of methods with updated column chemistry is an inexpensive alternative to synergize costs with problematic methods or outdated USP methods. As already seen, automation with AMDS helps cut down on the redevelopment time. *IS*™ columns help with introducing speed while maintaining peak shape and robustness with little regard to peak capacity. However if an R&D lab needs to develop more complex methods of high peak count such as peptides or natural products, *IS*™ columns are not a viable option. Only newer advances in LC technology may be a long term cost effective option to save on analysis time.

Recently a new field of LC emerged. ACQUITY UPLC™ (Ultra Performance Liquid Chromatography) offers the ability to maintain sensitivity, speed and resolution with methods that have a high peak count. Waters ACQUITY UPLC™ has increased performance to pressures of 15,000 psi which allows for the higher peak capacity. This technique is still new and many questions have been asked about how to transfer HPLC methods to ACQUITY UPLC™ methods. In the following example (figure 6), a practiced HPLC method on a ginger root extract will be optimized in a semi-automated environment for a better HPLC method and then transferred to ACQUITY UPLC™ to yield a method that is not only improved but also optimized with flow rates suitable to be run by mass spectroscopy without flow splitting.

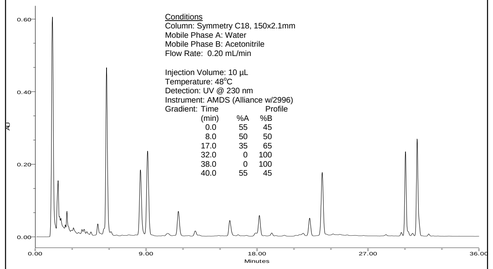


Figure 6: Original ginger root HPLC method as per *Ref: Xian-guo He et al, J. Chromatogr. A 796 (1998) 327-334.*

AMDS Set-up

Analytical Goals:

Priority: Resolution more important than run time
Resolution: > 2.0
Run time: < 10.0 minutes
Separation: Gradient preferred
Max. Pressure: ~3000psi
Min. t0: 2 times t0 (typical for USP methods)

Based on the previous conditions used in the original method, water and acetonitrile are still used at 230nm. A different column was substituted knowing the end method was to be compatible with an ACQUITY UPLC™ system such that the column would be able to withstand the very-high pressures (12,000-15,000psi).

The complexity of this sample and the many small detected peaks may pose a problem for AMDS in a fully automated sense. To aid in our optimization goal, the AMDS decision manager was set-up to allow user intervention at the end of each sample set. This allows the user to halt the development process, select the peaks of interest and proceed with re-automating the prediction process.

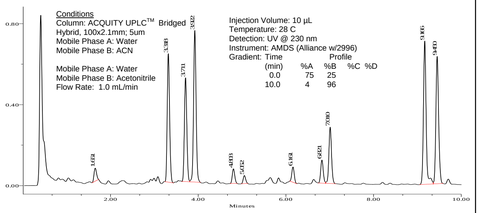


Figure 7: AMDS resulting method as predicted by Drylab. **Developed in ~4hrs unattended** **Note that the method gradient conditions are now linear as opposed to a multi-step gradient.*

Manipulating Simulations in Drylab

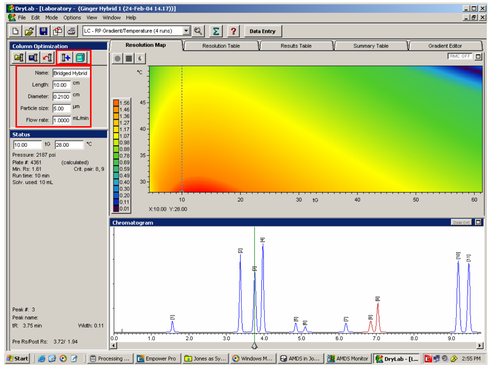


Figure 8: Drylab Resolution Plot of ginger root peaks of interest.

This is the resulting Drylab Resolution Plot which can be used to modify the conditions needed to simulate ACQUITY UPLC™ conditions. ACQUITY UPLC™ follows traditional LC theory which allows this to be possible. For an instrument to instrument transfer, Drylab plots can be modified with the new instrument parameters such as dwell, extra-column volume, and column parameters in the highlighted spaces above. Once entered, analysis simulations can be predicted for the new instrument of choice. It should be noted that having the proper extra-column volume entered in Drylab is key to having accurate predictions of resolution. The ACQUITY UPLC™ systems have considerable less band broadening than traditional HPLC and quality simulations must take this into account.

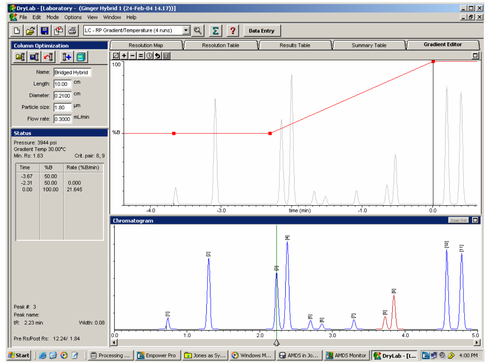


Figure 9: Drylab Gradient Editor

Other modifications with Drylab may be necessary besides modifications to instrument performance parameters to maintain resolution and selectivity. Drylab's gradient editor allows the user to simulate gradient time points to fully optimize a separation. In this example an initial hold was added to speed analysis time and close any unwanted resolution. It may also be noticed in the resolution plot that changes in temperature may be used to help optimize the separation.

UPLC Ginger Root Results

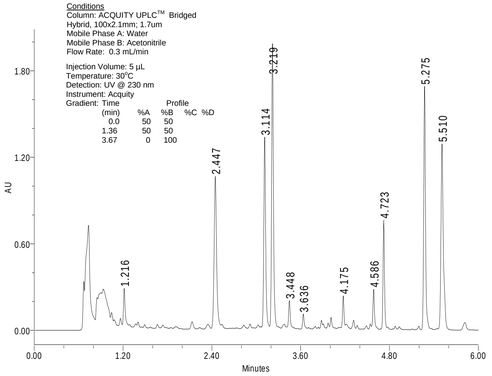


Figure 10: ACQUITY UPLC™ results. **Determined and performed in just minutes using the data gathered by AMDS and Drylab predictions.**

The ACQUITY UPLC™ results yielded a 6 fold improvement in run time from the original 36-40 minute method. The sensitivity is a 2 fold increase with half the injection volume than that of the HPLC method at 10minutes. Gradient recovery time is also decreased when using ACQUITY UPLC™ because of the small amount of dwell time of the system such that injection to injection is faster.

Discussion and Conclusions

In conclusion, automation by way of AMDS assisted with small Drylab interface time can aid in an efficient approach to development or re-development of methods for fast separation applicability. Using AMDS allows the user to run the development process unattended with a systematic SOP-like approach with automatic upload to Drylab2000 in a matter of hours. Automatic uploading of data saves time instead of watching injection to injection attempts of trial by error approaches. The automatic upload saves time with the complexities with tasks of importing and exporting data. For long term profitability, following a process as described above facilitates the potential gains which could be produced by either chemistry improvements such as *IS*™ column technologies or by instrumental advances brought by ACQUITY UPLC™.

It should be obvious what this could mean for a lab as proven according to the presented data, but how does effect a company as a whole? In a QC environment where many labs are now under cost analysis investigations to save money by limiting solvent consumption, waste disposal and downtime due to poor methods. Solving these and many other problems by just staying updated with what technology can offer can trickle down to faster batch turn around time, higher batch approval percentage, and eventually less inventory needed on hand. For Instance, what used to be a 5 day inventory of product base because of lengthy analysis times can now be a 2 day inventory base because a lab spent little time to re-develop and cross-validate methods that are now 3-6 fold less analysis time. Manufacturers know how synergized inventory could save millions, and this process serves as an efficient means to get there.

The savings for an R&D environment is more subtle monetary speaking but much more noticed with flexibility with time management with multiple projects. It would mean less complaints and struggle between R&D and QC. The thousands of simulations that the Drylab resolution plot reaches analytical goals that meets your business needs, but also provides insight to chromatographic behaviors and trends.

Saving time and money in these areas can help generics and/or contract labs produce for more for less. These processes can give any generic/contract labs that edge to win bids on working with drugs coming off patent. The systematic approaches build confidence as well as alliances with "Big Pharma" which can lead to more interaction and profits.