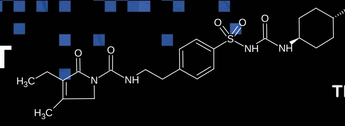
A METHOD DEVELOPMENT APPROACH TO ACHIEVE AN IMPURITY PROFILE FOR IMPURITIES PRESENT IN A BULK DRUG SUBSTANCE USING SUB 2µM POROUS PARTICLE LC COMBINED WITH UV-MS



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Scouting → ACQUITY UPLC, ACQUITY SQD, Empower 2 CDS

Data Analysis → Empower 2, Custom Calculations, Custom Reporting

Optimization

INTRODUCTION

Impurity profiling of pharmaceutical drug substances or dosage forms require methods involving high sensitivity and specificity as well as desirable analysis times. Liquid chromatography and mass spectrometry have become essential tools in the analysis and characterization of drug impurities, with analysis in the 30 minute to 1 hour time frame. In this presentation an efficient method development screening process was employed utilizing short UPLC columns and a generic gradient to fast track the method development screening time. The process takes advantage of the high chromatographic efficiency of sub 2µm particle (UPLC®) technology to deliver rapid method scouting. The use of short columns allowed many column chemistries to be screened quickly in an automated manner using a column manager capable of addressing four columns. Once the best column chemistry was selected, further optimization for resolution was achieved by varying gradient slope and temperature on the longer column dimension. Employing photodiode array detection coupled with single quadrupole MS facilitated the peak tracking as the conditions were varied. The use of specific labeling custom fields in the chromatographic data system allowed for the creation of custom reports to help expedite the mining of the resulting data.

METHODOLOGY

Chromatographic Tools

- ACQUITY UPLC w/ column manager
- ACQUITY PDA coupled to ACQUITY SQD
- Configuration #1 (figure 1)

Four ACQUITY UPLC chemistries 2.1 x 50 mm, 1.7 µm

- AQUITY UPLC BEH C18
- ACQUITY UPLC BEH Phenyl
- ACQUITY UPLC BEH Shield RP18
- ACQUITY UPLC HSS T3

Solvents

- Acetonitrile
- Methanol

Buffers

- Low pH (pH 3 Ammonium Formate)
- High pH (pH 10 Ammonium Bicarbonate)

Approach

- Short Columns for Rapid Screening
- 5 minute gradients from 5-95% organic
- Modify gradient as dictated by sample hydrophobicity
- Temperature at 35°C
- Flow rate of 0.6 mL/min

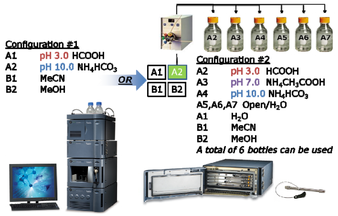


Figure 1: Configurations of ACQUITY UPLC for Method Development

METHOD SCOUTING MANAGEMENT

UTILIZING EMPOWER 2 CDS

The Empower 2 CDS was the essential cog allowing a streamlined method scouting strategy. Figure 2 was an example Sample Set method utilizing Empower 2 for method scouting. Various functions highlighted below in green such as "column condition", "equilibrate", and "wet prime" facilitated an increase in injection to injection readiness and system stabilization. The "Column Position" field allowed for the synergy of multiple instrument methods. (i.e. Four methods can now be used as opposed to four methods for each column position)
The custom fields highlighted below in red for "column type", "solvent type" "Buffer Type" and "pH" were key because they were used to aid the visualizing and mining of the data set after it is acquired.
We typically use the same solvents or set of columns, therefore the custom field was created as drop down menu of fields with pre-filled entries. This increased the selection of the correct column and solvent for a particular injection without transcription errors

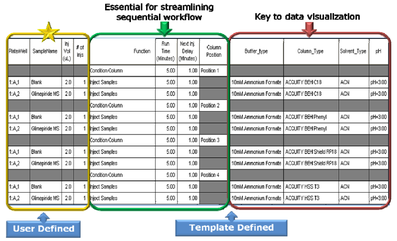


Figure 2: Example Sample Set template in Empower 2 CDS

CHROMATOGRAPHIC OBSERVATIONS

The method scouting approach that was implemented exploited the selectivity effects when changing pH, column ligand, and organic modifier. In many cases selectivity is not the only effect observed. Changes in peak shape can occur and hence overall sensitivity can be optimized.

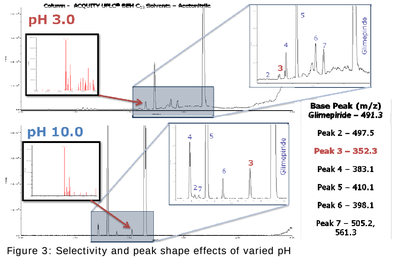


Figure 3: Selectivity and peak shape effects of varied pH

ASSESSING PEAK ATTRIBUTES

Examination of a homogeneous peak vs. a non-homogeneous peak illustrated the *purity angle*, in green, is beneath the purity threshold, in blue. The peak is spectrally pure in this instance, essentially meaning no co-elution. With a non-homogenous peak, the point of maximum difference in the spectra can be denoted, shown below with the "M" indicating a co-elution. Multiple purity passes will assist in determining multiple co-elutions

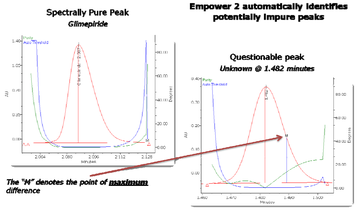


Figure 4: Peak Purity plot as determined in Empower 2 CDS

IMPORTANCE of MASS SPECTROMETRY

Tracking peaks by UV during the method development process may be difficult as the spectra of related substances can be similar. With MS data, the peaks can now be easily tracked as well as any additional peaks invisible to UV detection.

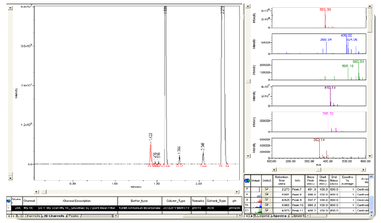


Figure 5: MS spectra with chromatogram attributes in same window

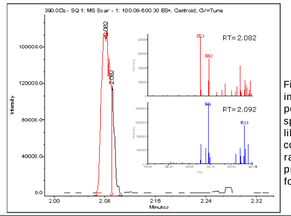


Figure 6: MS integrated trace of peak X. The MS spectra identified the likelihood of co-eluting peaks rather than the presence of adduct formation.

MINING THE DATA

CRITERIA FOR DATA ANALYSIS

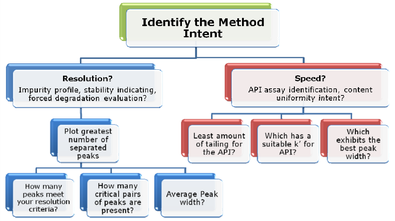
The scouting experiment and acquiring all of the data is completely automated. Without the proper data analysis and management tools, evaluation of the data is quite burdensome and time-consuming. The system suitability option allowed for the evaluation of:

- Resolution values per peak, Retention of API, Various peak widths, Tailing factors, Area%, S/N

The entire matrix of results to make the proper method development decision totaled 14 runs, 7 separate decision factors, up to 20 peaks, and 2 modes of detection
Data to evaluate:

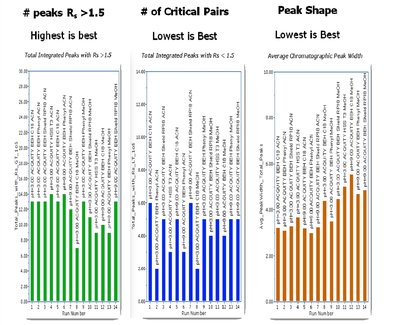
- 1 run = 280 resulting values
- 14 runs = 3920 resulting values

To help focus us during data evaluation process, it is important to keep in mind the intent of the method. Is it resolution or speed or perhaps a combination of both with one being of higher priority.



EMPOWER 2 CUSTOM REPORTING

Plots help distinguish between multiple variables that represent different separation conditions by identifying general separation criteria or properties for the peaks in each chromatogram. These particular plots were useful for most evaluations and particularly useful for the impurity profiles, stability indicating methods, and multiple component analyses.



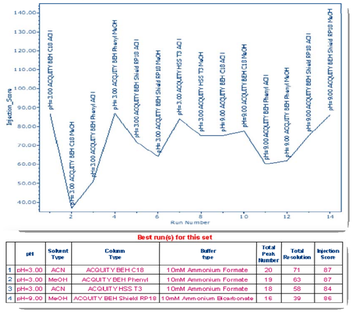
EMPOWER 2 CUSTOM CALCULATIONS

An "Injection Score" was used to assess all the resulting plots. It was an estimation of conditions which were suitable to explore for further optimization. In this this particular example, the injection score equation included factors about:

- Total peaks found
- Total peaks above 1% area
- Run time
- Average peak width
- Separation space

**Injection score equations are recommended to include a weighted relationship of the user's goals and criteria*

Chromatograms were easily located to verify the best case scenario

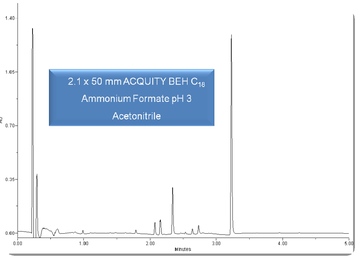


	pH	Solvent Type	Column Type	Buffer Type	Total Peak Number	Total Re-solution	Injection Score
1	pH=3.00	ACN	ACQUITY BEH C18	10mM Ammonium Formate	20	71	87
2	pH=3.00	MeOH	ACQUITY BEH Phenyl	10mM Ammonium Formate	19	63	87
3	pH=3.00	ACN	ACQUITY HSS T3	10mM Ammonium Formate	18	58	84
4	pH=9.00	MeOH	ACQUITY BEH Shield RP18	10mM Ammonium Bicarbonate	16	39	86

Figure 7: Injection Score summary

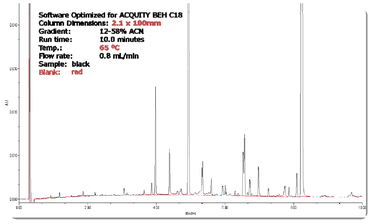
RESULT FROM EMPOWER DATA MINING

Result based on Empower 2 data interpretation with Total Number of Peaks and Injection Score, the conditions to optimize for the glimepiride impurity profile are shown below.



OPTIMIZATION PROCESS

The optimization process included variations of gradient slope, temperature, and column dimensions. The guidance of simulation software (Drylab® 2000 Plus, Molnar-Institut, Germany) assisted optimization of the chromatogram with the most efficient process. Upon four injections which were strategically suggested by the software, the input of retention times and area% of each peak of interest was performed. The MS and PDA spectral information aided the peak tracking. Properly tracked peaks increased the predictive ability of the simulation software resulting in a more successful confirmation result of predicted optimal conditions.



CONCLUSION

More efficient method development using a systematic approach

- **Allows more analytical space to be covered**
- **Leads to more robust and better methods quicker**
- **Increases laboratory productivity**

Implementing this approach requires

- **Range of column chemistries**
- **System designed to provide both resolution and productivity**
- **Informatics that aids in data interpretation and sharing**

Benefits

- **Overall time savings of approx 6X**
- **Less consumption, Less waste**

UPLC Method Development Protocol 2.1 x 50 mm, 1.7 µm	
pH 3/ acetonitrile x 4 columns	**Time**
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
pH 3/ methanol x 4 columns	
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
pH 10/ acetonitrile x 3 columns	
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
pH 10/ methanol x 3 columns	
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
pH 3 scouting time	272 minutes
pH 10 scouting time	204 minutes
SCOUTING TIME	**476 min**
TOTAL SCOUTING TIME	**7.9 HOURS**

EQUITY HPLC Method Development Protocol 4.6 x 150 mm, 5 µm	
pH 3/ acetonitrile x 4	**Time**
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
pH 3/ methanol x 4	
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
pH 10/ acetonitrile x 3	
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
pH 10/ methanol x 3	
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
pH 3 scouting time	1576 minutes
pH 10 scouting time	1152 minutes
SCOUTING TIME	**2728 min**
TOTAL SCOUTING TIME	**46.0 HOURS**

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