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A New, Single HPLC Method for the Analysis and Regulation of a Multi-Component Pharmaceutical Formulation and Related Substances

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Abstract

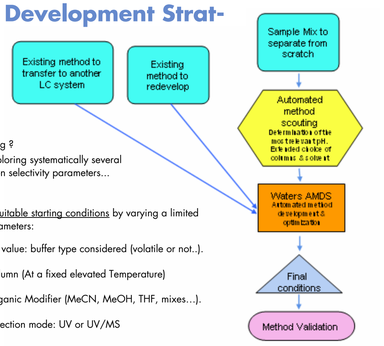
The current USP lists at least four different methods for the regulation and control of formulations containing triamterine, hydrochlorthiazide, and related substances. These methods include different column types and dimensions, different pH's and different buffers; none of which are compatible with MS detection. Other examples can also be found in the USP and the literature in general where multiple methods are sometimes necessary to accomplish similar goals. When faced with having to re-develop and validate methods like these for more efficiency and productivity, robustness and MS compatibility, most analysts refrain because they do not have the time to invest due to other tasks at hand. In this work we will describe one method on one column that accomplishes all of the required tasks, and how it was derived with a minimal time investment. Various column chemistries were scouted, and the method was re-developed and validated in an automated fashion with a minimum of operator intervention. The software and instrument tools necessary to accomplish this task will is described, as well as the validation data. Results from the method in actual practice is also be presented. And finally, we show that our approach can be universally applied by presenting a protocol that analysts can use to simplify and improve the efficiency, productivity and robustness of other methods in spite of their time constraints.

Discussion

May of the LC methods in the USP are older methods developed and validated with sometimes decades-old technology. However, many analysts will use the methods as is, because little or no time exists to redevelop, optimize, validate and/or demonstrate equivalency. Our goal is to utilize a strategy that consists of methodical column scouting, automatable method optimization, and a standardized validation SOP accomplished through the use of software, templates and wizards that easily guide the user through the entire process. A more efficient automatable approach increases productivity, and enables analysts to redevelop and validate methods to take advantage of new technology in a fraction of the time normally needed.

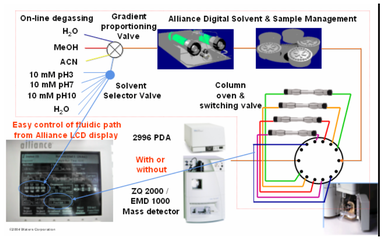
This poster describes an approach that redevelops (from scratch) several related methods into a single, more robust method in a matter of days that takes advantage of new technology to improve productivity by 2-5X. This strategy can be applied to any method development challenge, whether the goal is to develop a new method, or to optimize an existing one.

Method Development Strat-



- What is scouting ?
  Scouting is exploring systematically several carefully chosen selectivity parameters…
- Why ?
  To determine suitable starting conditions by varying a limited number of parameters:
  - pH value: buffer type considered (volatile or not..).
  - Column (At a fixed elevated Temperature)
  - Organic Modifier (MeCN, MeOH, THF, mixes…).
  - Detection mode: UV or UV/MS

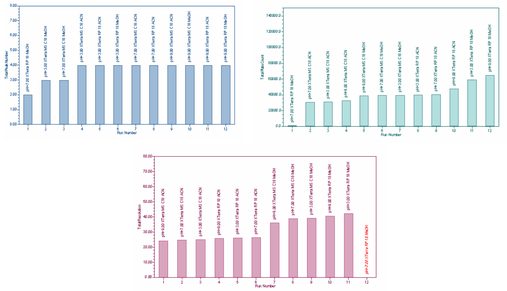
Column Scouting System Schematic



Column Scouting Software Template

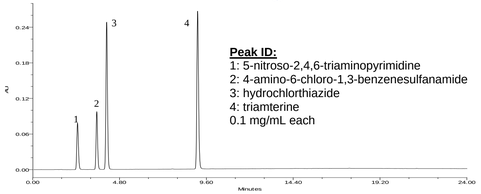
	Vial	SampleName	Inj Vol (ul)	# of Injs	Function	Method Set / Report Method	Run Time (Minutes)	Next Inj Delay (Minutes)	Purge Flow (ml/min.)	Column Position	Column_Type	pH	Solvent Type	Buffer_type
1					Wet Prime	Prime_A1	2.00		10.00					
2					Condition Column	Scout_A1_ACN	16.00			Position 1				
3					Equilibrate	Scout_A1_ACN	7.00	0.00		No Change				
4	30	Sample Mix	10.0	1	Inject Samples	Scout_A1_ACN	18.00	6.00			XTerra MS C18	pH=3.00	ACN	10mM Ammonium Formate
5					Equilibrate	Scout_A1_MeOH	10.00	0.00		Position 1				
6	30	Sample Mix	10.0	1	Inject Samples	Scout_A1_MeOH	24.00	6.00			XTerra RP 18	pH=3.00	MeOH	10mM Ammonium Formate
7					Equilibrate	Column_Rinse	10.00	0.00		No Change				
8					Condition Column	Scout_A1_ACN	16.00			Position 2				
9					Equilibrate	Scout_A1_ACN	7.00	0.00		No Change				
10	30	Sample Mix	10.0	1	Inject Samples	Scout_A1_ACN	18.00	6.00			XTerra MS C18	pH=3.00	ACN	10mM Ammonium Formate
11					Equilibrate	Scout_A1_MeOH	10.00	0.00		Position 2				
12	30	Sample Mix	10.0	1	Inject Samples	Scout_A1_MeOH	24.00	6.00			XTerra MS C18	pH=3.00	MeOH	10mM Ammonium Formate
13					Equilibrate	Column_Rinse	10.00	0.00		No Change				
14					Wet Prime	Prime_A4	2.00		10.00					
15					Wet Prime	Prime_A2	2.00		10.00					
16					Condition Column	Scout_A2_ACN	16.00			Position 1				
17					Equilibrate	Scout_A2_ACN	7.00	0.00		No Change				
18	30	Sample Mix	10.0	1	Inject Samples	Scout_A2_ACN	18.00	6.00			XTerra RP 18	pH=7.00	ACN	10mM Ammonium Acetate
19					Condition Column	Scout_A2_MeOH	22.00			Position 1				
20					Equilibrate	Scout_A2_MeOH	10.00	0.00		No Change				
21	30	Sample Mix	10.0	1	Inject Samples	Scout_A2_MeOH	24.00	6.00			XTerra RP 18	pH=7.00	MeOH	10mM Ammonium Acetate
22					Equilibrate	Column_Rinse	10.00	0.00		No Change				
23					Condition Column	Scout_A2_ACN	16.00			Position 2				
24					Equilibrate	Scout_A2_ACN	6.00	0.00		No Change				
25	30	Sample Mix	10.0	1	Inject Samples	Scout_A2_ACN	18.00	6.00			XTerra MS C18	pH=7.00	ACN	10mM Ammonium Acetate
26					Condition Column	Scout_A2_MeOH	22.00			Position 2				
27					Equilibrate	Scout_A2_MeOH	10.00	0.00		No Change				
28	30	Sample Mix	10.0	1	Inject Samples	Scout_A2_MeOH	24.00	6.00			XTerra MS C18	pH=7.00	MeOH	10mM Ammonium Acetate
29					Equilibrate	Column_Rinse	10.00	0.00		No Change				
30					Wet Prime	Prime_A4	2.00		10.00					

Data Mining For Best Separation



Custom reports can be used to summarize the column scouting data so that the most promising separation can be picked out at a glance. The separation shown below was chosen by quickly examining the above plots instead of tediously reviewing each individual separation.

Column Scouting Results



At first glance, it may appear that the separation is complete. But while this separation looks good, is it truly the optimum? Can it be performed in less time? Is it robust? Further optimization could be time consuming; however by utilizing an automated system further optimization an be done quickly and easily.

Automated Method Development System

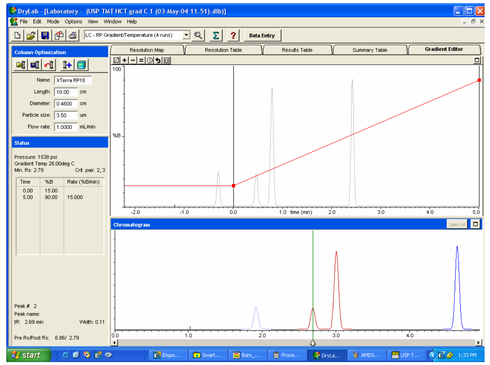
Waters AMDS is:

- Independent or complementary to Column Scouting
- Performed on the same Alliance/PDA/ Empower System
- For both Method Development and Method Optimization

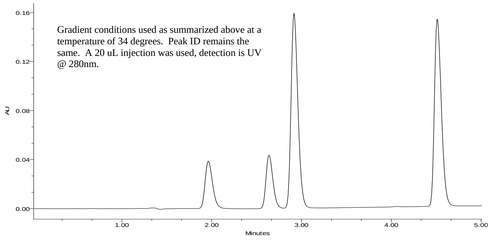
Waters AMDS Wizard includes :

- A toolkit to set up the instrument using LC Resources DryLab® as a prediction engine
- Waters Automated Peak Tracking Algorithm
- An iterative Decision Manager process

AMDS Predicted Optimum Separation

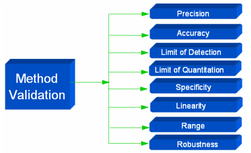


AMDS Predicted Separation Verification

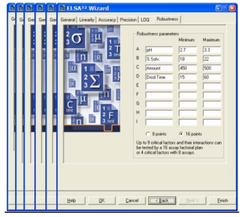


In a few short hours, AMDS has re-optimized the separation automatically, unattended. The result is a more robust separation accomplished in half the time. The corresponding USP method approaches twenty minutes, with broad, tailed peaks that ultimately effect accuracy and precision. Further study of the DryLab resolution maps yield information about method robustness, and its suitability for method validation.

Method Validation



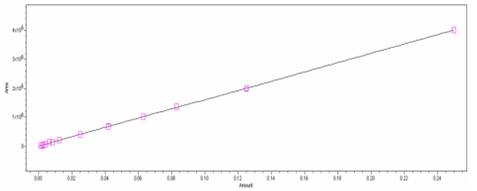
Method validation is carried out to ensure that the method accomplishes it's intended purpose. Over the course of this process, several performance parameters must be measured and documented. The actual parameters investigated depends upon the type of method and what it is being used for.



Method validation can also be carried out according to established SOP's using wizard-driven software templates. Using relational database view filters, Elsa32 software captures the data in a traceable format and performs the standard statistical calculations needed to make objective decisions regarding method validation.

Method Validation Examples: Linearity

Linearity must be performed over a minimum of five levels. The data generated below was from triplicate injections over 12 levels, spanning the expected range for the assay of both content and impurity/related substances/degradation studies, over three orders of magnitude. Plot is for triamterine; the other three components yielded similar results summarized below.



Component	R^2	Equation
NTAP	0.99990	$Y = 4.76e^6 + 3.18e^3$
ACBS	0.99998	$Y = 3.72e^6 + 5.26e^3$
HCT	0.99989	$Y = 1.00e^7 + 1.26e^4$
TMT	0.99990	$Y = 1.60e^7 + 1.28e^4$

Limit of Quantitation/Detection

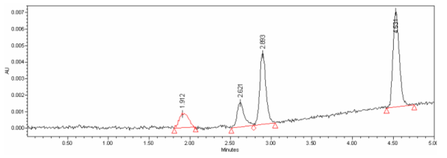
Quantitation and detection limits were calculated from the lowest three levels of the linearity data according to the formula:

Limit = A (Standard deviation of the response/Slope of calibration cure)

Where A = 10 for the quantitation limit, and 3.3 for the detection limit.

	Level 1 Response	Level 2 Response	Level 3 Response	Level 4 Response	Level 5 Response
1	8164	13585	21296	42395	61740
2	8130	13785	20936	42467	63528
3	8734	13195	21220	41976	62152
Mean	8343	13522	21151	42279	62473
STDev.	339.3	300.0	189.8	265.1	936.3

Average Standard Deviation:	406.1
Slope of Calibration Curve:	4760000
Limit of Detection	0.00025 mg/mL
Limit of Quantitation:	0.00085 mg/mL



System Suitability

Component	Avg. Area	%RSD Area	Avg. Rt.	%RSD Rt.	Avg. Rs.	%RSD Rs.	Avg. N	%RSD N
NTAP	298826	0.5	1.926	0.2	-----	-----	1291	0.9
ACBS	239073	0.5	2.622	0.1	3.92	0.286	5748	1.2
HCT	642003	1.0	2.889	0.1	1.97	0.416	7149	1.2
TMT	1018352	0.8	4.535	0.1	11.77	0.499	17997	1.4

System suitability was performed before and after running the linearity/calibration data. Six replicate injections at a concentration of 0.63 mg/mL were made. Shown above are the post run results. The pre-run results were virtually identical.

Not shown are the PDA spectral results investigated for specificity and accuracy. All peaks were determined to be single components, free from interferences when samples of the drug product were run.

Conclusion

- **Several older USP methods were re-developed into a single, shorter, more robust method.**
- **Use of software templates for method scouting and validation and Automated method development/optimization saves significant time and can provide unique robustness information.**
- **Time saving tools that improve efficiency and throughput**

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