UTILIZATION OF UPLC/MS AND EMPOWER 2 CDS FOR EFFICIENT METHOD DEVELOPMENT OF AN IMPURITY PROFILE OF SIMVASTATIN

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INTRODUCTION

Simvastatin, a well known cholesterol-lowering prescribed class of statin, when taken orally hydrolyzes to the β-hydroxy acid form which acts as an inhibitor of the 3-hydroxy-3-methylglutaryl-coenzyme, A (HMG-CoA) reductase enzyme involved in the in vivo synthesis of cholesterol[2]. Recently the patent rights for production of simvastatin have expired allowing it to be manufactured by generic pharmaceutical companies. There are several methods reporting the analysis of simvastatin and the related impurities. Two official methods are reported in the European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP) utilizing HPLC gradient methodology [1,2]. These methodologies are typically time consuming with analysis times in excess of 30 minutes. In order to address the business needs of a generic pharmaceutical company, a faster methodology is required that does not compromise analytical quality.

In this study, the application described will demonstrate the utility of Ultra Performance LC® (UPLC) technology and Empower 2™ software to aid in the efficient method development process of impurity profiles for pharmaceutical drug entities. The HPLC method for simvastatin has been redeveloped on UPLC to be compatible for MS. The analytical goals were to meet the requirements stated in the USP 30 - NF 25 monograph for simvastatin drug substance and drug product chromatographic purity and assay identification. Empower™ 2 custom reporting, custom fields, and spectral analysis were used in streamlining the decision making process during method development. Coupling this technique with single quadrupole MS with electrospray positive/negative ionization detection will aid in the discovery of any impurities with no chromaphores. The pre-validation of the methodology was performed with the assistance of Method Validation Manager (MVM) to verify method conversion equivalency. A series of validation tests commonly referred to as the "SLAP" technique will verify specificity, linearity, accuracy and precision and prove method applicability for the drug substance assay. By performing this initial pre-validation, the methodology is best prepared for the next phase which in many cases would be a complete validation.

EXPERIMENTAL

Materials:
United States Pharmacopoeia Simvastatin RS (Rockville, MD); United States Pharmacopoeia Lovastatin RS (Rockville, MD);

Reagents: Acetonitrile Optima; Fisher Scientific (Fairlawn, NJ); Lot#050580. Ammonium Acetate and acetic acid; Sigma-Aldrich (St. Louis, MO); .

CONCLUSION

- An efficient method development screening process was employed utilizing short UPLC columns and a generic gradient to fast track the method analysis screening time
- The use of short UPLC columns allowed many column chemistries to be screened quickly in an automated manner using the ACQUITY column manager
- The use of specific labeling custom fields in Empower 2 CDS allowed for the creation of custom reports to help expedite the mining of the resulting data
- Orthogonal data sets such as MS were beneficial in best confirming existence of unknown peaks. The application of MS ES pos/neg allowed for the facile identification of impurities without chromophores
- Method validation tests can be performed easily and efficiently when assisted by MVM. Preliminary "pre-validation" testing can be used for an initial assessment of the method's performance and suitability for it's intended purpose. In addition, the collected data will be re-purposed in the final complete validation study.

References

1. European Pharmacopoeia, 4th Edition (2002) Council of Europe, Strasburg Cedex
2. United States Pharmacopoeia, USP30–NF25 Page 3179 Pharmacopeial Forum : Volume No. 32(1) Page 141

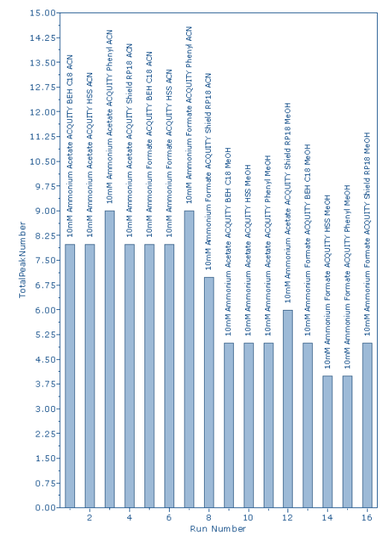
METHOD SCREENING

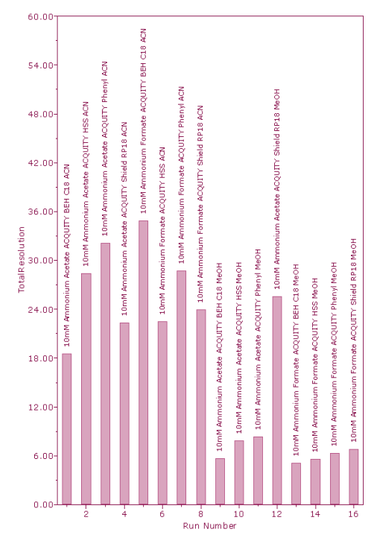
Mining the Data

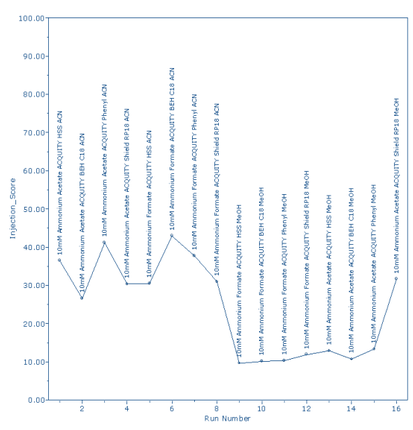
Empower™ 2 CDS was employed to "mine" the data without the need for manual review of the numerous injections in whole data sets. Simple drop down menus within the CDS allow for the rapid review of the effects of buffer type, solvent, pH, and column type. Interpretation of the method scouting data of simvastatin and related impurities in conjunction with these custom reporting features resulted in an easy to read summary report. For each condition, the report displays the total # of peaks detected and the total resolution. These values were automatically calculated to determine an "injection score". Injection scores can be configured to account for any chromatographic criteria that the method development group uses to make "next step" decisions.

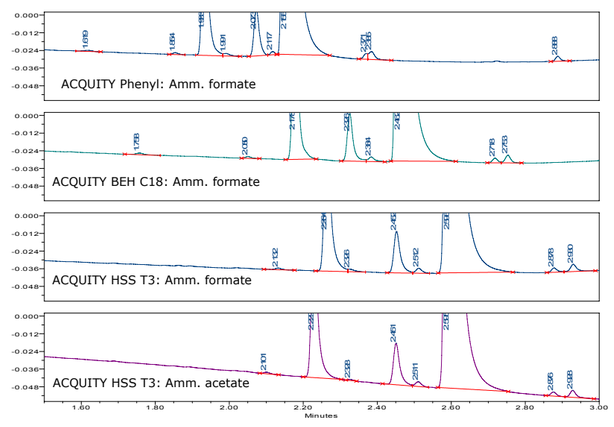
UPLC Method Screening Conditions

Instrument:	Waters® ACQUITY UPLC® System
Column:	50 x 2.1mm, 1.7µm (1.8µm for HSS)
Column 1:	ACQUITY UPLC® HSS C_{18} Column
Column 2:	ACQUITY UPLC® BEH C_{18} Column
Column 3:	ACQUITY UPLC® Phenyl Column
Column 4:	ACQUITY UPLC® Shield RP_{18} Column
Mobile Phase:	A1: 15mM Ammonium formate pH 4.0
	A2: 15 mM Ammonium acetate, pH 4.0
	B1: Acetonitrile
	B2: Methanol
Gradient:	linear 2-100% B1/ 3 min (ACN)
	linear 2-100% B1/ 5 min (MeOH)
Flow Rate:	0.80 mL/min
Temperature:	30°C
Injection Volume:	3 µL; PLUNO injection mode
Detection:	ACQUITY PDA w/ λ extracted at 238 nm





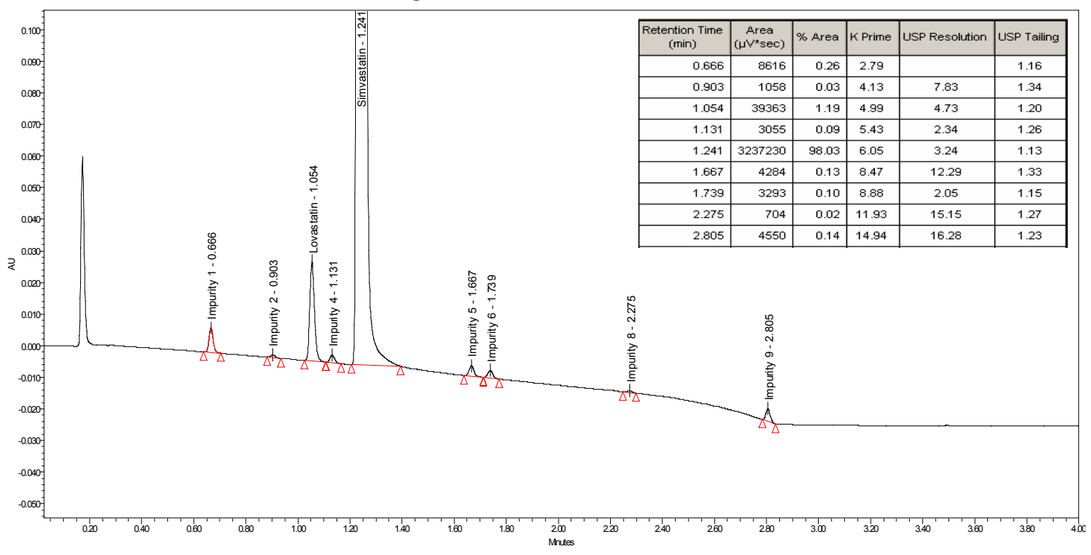




Customized summary plots can either be bar charts or line plots. The summary plots indicated that the phenyl column with the ammonium formate buffer would yield the best average results. However the phenyl column had difficulty resolving peaks RT=2.371 minutes and RT=2.385 minutes. A review of the four chromatograms giving the greatest number of peaks and highest total resolution number (left), confirmed that the conditions for the ACQUITY HSS C18 column with ammonium acetate resulted in the best resolution between the critical pairs of peaks RT=2.229 minutes and RT=2.328 minutes.

METHOD OPTIMIZATION

The UPLC method was optimized for all of the impurity peaks in the sample identified with similar spectra to that of simvastatin and not present in the blank. An experimental design of four injections were performed including two different linear gradient slopes (5 minutes and 10 minutes) and two different temperatures (30°C and 50°C) to optimize the LC separation. The resulting data was collected and entered into chromatographic modeling software. Conditions included the ACQUITY HSS C18 column 2.1mm x 50mm; 1.8µm to maximize speed and maintain chromatographic resolution greater than 2.0 between the critical pair. The flow rate was 0.8ml/min. The final method conditions resulted in the chromatogram below.



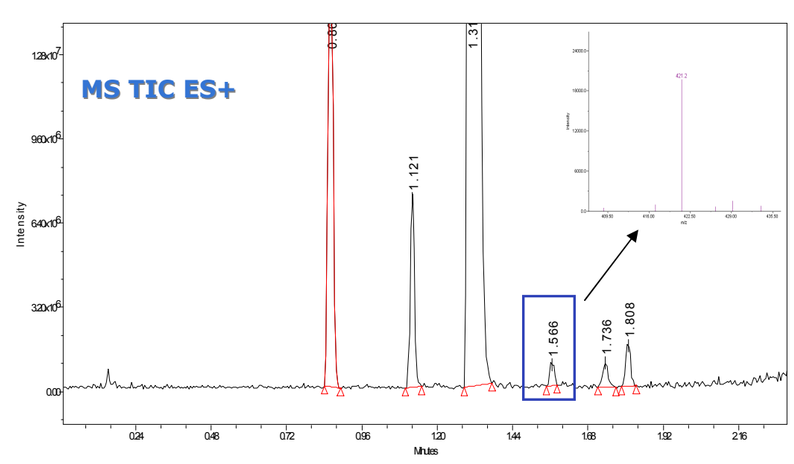
Retention Time (min)	Area (µV*sec)	% Area	K Prime	USP Resolution	USP Tailing
0.666	8616	0.26	2.79		1.16
0.903	1058	0.03	4.13	7.83	1.34
1.054	39363	1.19	4.99	4.73	1.20
1.131	3055	0.09	5.43	2.34	1.26
1.241	3237230	98.03	6.05	3.24	1.13
1.667	4284	0.13	8.47	12.29	1.33
1.739	3293	0.10	8.88	2.05	1.15
2.275	704	0.02	11.93	15.15	1.27
2.805	4550	0.14	14.94	16.28	1.23

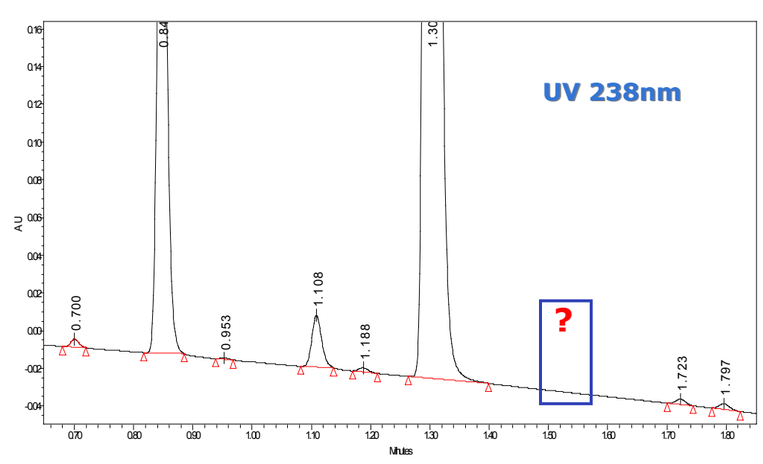
Final Method UPLC/MS conditions

LC System:	Waters® ACQUITY UPLC® System
MS Instrument:	Waters SQD ES±
Column Dimensions:	2.1 x 50 mm, 1.8 µm
Column 1:	ACQUITY UPLC HSS C_{18} Column
Column Temp:	40 °C
Flow Rate:	800 µL/min.
Mobile Phase A:	15 mM Ammonium Acetate, pH 4.0
Mobile Phase B:	Acetonitrile
Gradient:	52% B to 100%B over 2.5 min. with a 1.5min hold at 100%B
Scan Range:	100 — 1000
Scan Rate:	10,000 amu/sec
Cone Voltage:	20 V
Source Temp:	150 C
Desolvation Temp:	450 C
Desolvation Flow:	800 L/Hr
Total run time:	4.0 minutes
Inj to inj run time:	5.0 minutes

Orthogonal Data to Assist Specificity Determination

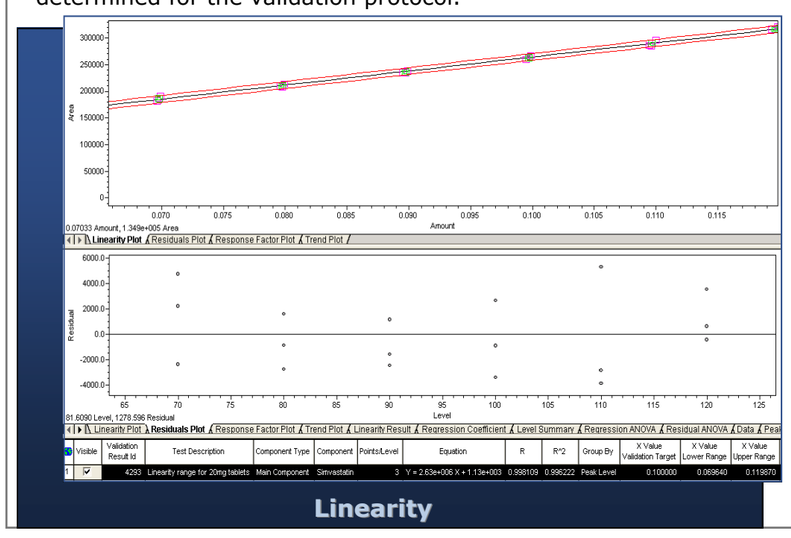
In this example, specificity is considered achieved if there is resolution between the spiked amount of lovastatin and the API, however any other interferences determined from blank diluent and present impurities, known or unknown will be taken into account. All blank injections were free of extraneous peaks. Acquiring MS positive/negative electrospray data and processing UV PDA spectra for peak purity allowed for the identification for many coelutions and interferences. The chromatograms below indicate the presence of a peak in the MS trace but not in the UV trace. UV 238nm was an extraction from the PDA 210-400nm range and the peak was not present. The MS ES– trace and peak purity values*(not shown)* did not indicate any interfering data, however the MS ES+ data not only indicated an unknown peak but facilitated peak tracking during the method optimization. *Future MS/MS and exact mass ID work will be initiated to determine peak origin.*

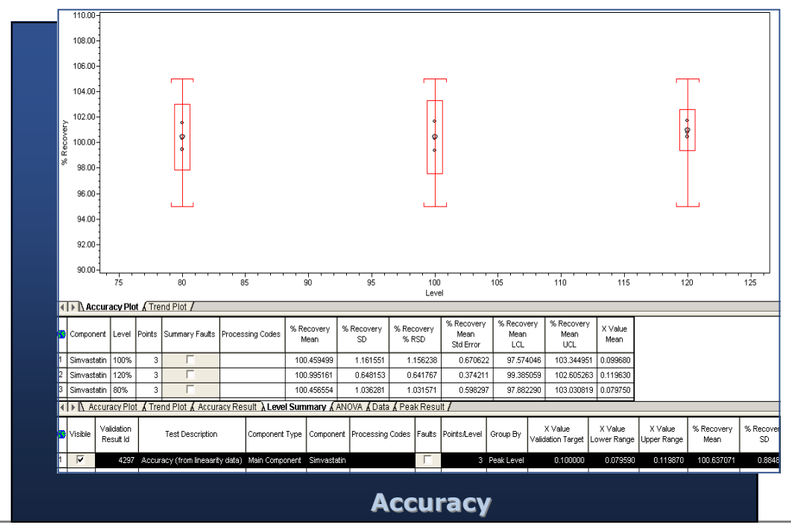


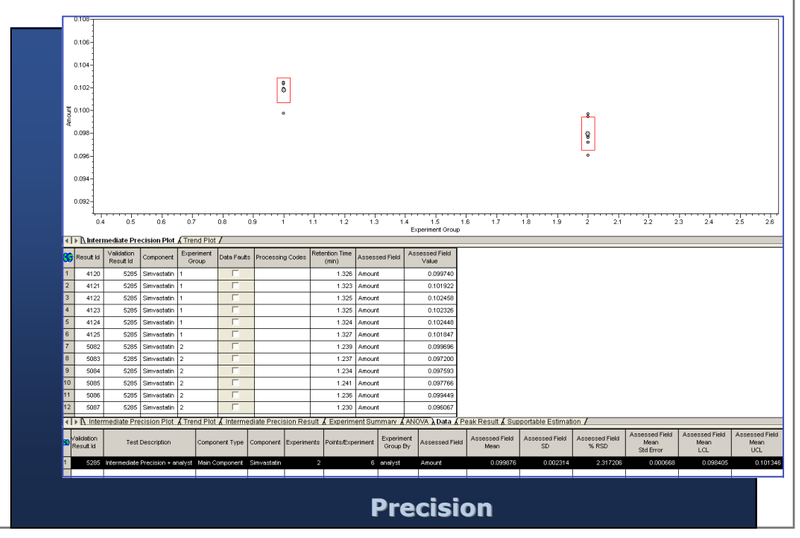


METHOD VALIDATION

A pre-determined set of initial validation test were performed to determine method applicability for the API simvastatin drug substance. The data was collected and processed using Empower™ 2 Method Validation Manager (MVM) such that the preliminary results could be combined with the results of a *future* full validation study if the criteria set passes specifications. Three qualification tests performed included linearity, accuracy, and precision. *(intermediate precision).* The criteria set for linearity was R^2 = 0.990 for 3 replicate sets with a relative response factor mean to be within a range of 0.900-1.100. It was determined that the linearity of 3 replicates across six levels of a 20mg tablet range was R^2 = 0.996 and the residual plot did not show any systematic error because of the observed random pattern. The The accuracy criteria was set to meet a %recovery range of 95% - 105% over 3 levels 80%, 100%, and 120% for the API. The %recovery mean for each level was 100.46%, 100.99%, and 100.46% respectively. In order to address precision, a second analyst assisted in testing six replicates of the standard preparation at 100%. The acceptance criteria was set for 2.0%RSD for the assessed "amount" field per analyst. The amount %RSD for analyst 1 and analyst 2 were 1.02% and 1.41%, respectively. Based on the resulting data calculated for precision in MVM, acceptance criteria for analyst variance can be determined for the validation protocol.







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