DEVELOPMENT FOR NON VOLATILE CONGERS IN DISTILLED SPIRITS BY UV AND MS

Rapid, unattended simultaneous UV and MS method development

John Palmer, John Kimmel, Sue D' Antonio, Lynne Marshall and Rita Steed

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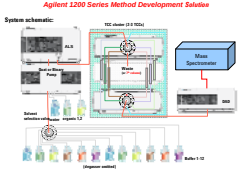
Introduction

LC method development can be a daunting task-especially when trying to find and identify unknowns. This is further complicated when separation methods must be developed to suit different types of detection or due to limits on mobile phase choices. Chromatographers often find themselves spending time developing separate methods with different mobile phases and columns. The congeners present in authentic bourbon represent this problem. These compounds are available in varying concentrations and the similarities in chemical make up make LC separation somewhat difficult. Use of LC/MS allows adequate detection and identification of the compounds without complete chromatographic resolution, however, not all laboratories posses a mass spec. A common occurrence in industry is that MS is located in R&D and UV detection is the rule in QC. The experiments illustrated in this poster were designed to quickly identify and track these compounds while testing the selectivity option provided by columns varying in hydrophobocity in one seamless development session.

Method Development Path

An HPLC, as listed below, incorporating an Agilent Method Development system with five different bonded phase columns (based on the same silica chemistry) installed, a Single Quad MS and a DAD detector was used to generate both MS data and DAD data simultaneously. Each sample injection was made under the same gradient conditions on each column. The MS detection permitted tracking of the compound retention for comparison to the UV chromatograms. The sequence of column and mobile phase selection was controlled by the Method Development System to provide unattended operation and data collection. The rapid experiments allow quick evaluation of chemical interactions on the columns and facilitated the discovery of method that satisfied the needs of LC/MS and LC/UV methods.

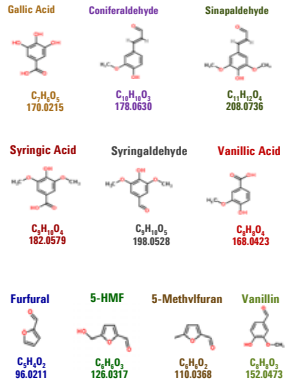


Experimental

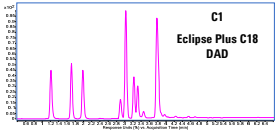
Sepration Conditions

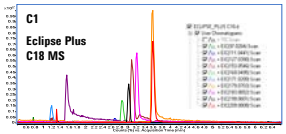
Agilent 1260 Quaternary pump
A (0.1% FA H_2O) B(0.1% FA MeOH)
Gradient 5%-95% B, 5minutes
Flow Rate 0.5mL/min.
G1367C ALS, Injection Volume 10 µL
Method Development System TCC 40° C
G1315C DAD, 254nm, 4nm, Ref Off
Single Quad MS
Columns 2.1 x 50mm. 1.8u
Eclipse Plus C18, Eclipse Plus Phenyl Hexyl
SB-C8, SB-AQ, SB-CN

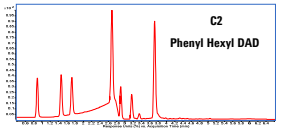
Bourbon Conger Structures and Mass Assignments

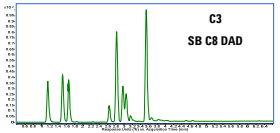


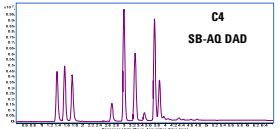
Results and Discussion

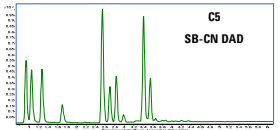












Chromatographic Sequence

C1-MS data identifies location on all components in sample, but C18 coes not provide complete resolution (conferaldehyde and sinapaldehyde coelute)

C2 – Phenyl Hexyl provides incomplete resolution

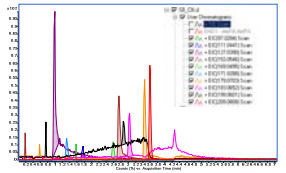
C3 – SB-C8 provides very similar separation to Eclipse Plus C18 with incomplete resolution

C4 – Improvement in middle of the chromatogram, but still incomplete resolution

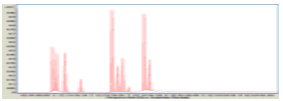
C5 – SB-CN provides complete resolution of all components.

Results and Discussion

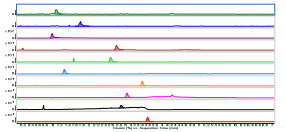
Chromatographic UV and Mass Spec Method



Individual component Injections



SB-CN TIC



TIC of Individual component Injections

Conclusions

This sample represents the challenges often encountered in the LC lab. Different compounds with differing response in either UV or MS and a mobile phase limited to one organic solvent and pH range. Rather than spending excess time developing separate methods for different labs this process developed one method applicable to both needs.

This was accomplished by first choosing a MS friendly mobile phase which was optimized for the C18 separation. This combination could be easily used for the MS analysis of many bourbon samples, but the lack of resolution of the two components would prevent labs limited to UV detection from using this method. The chemical interaction of the sample with the column/mobile phase was then changed by altering only the bonded phase chemistry. All columns use the same base silica to minimize that effect on the separation. Each column chemistry altered the separation. Some were better than others, but only one provided the best chromatographic method.

By employing 2.1 x 50mm length 1.8 micron columns in this method development path provided a good separation the required very little instrument time and produced a short method as well. This would help many development labs to better utilize lab resource and improve downstream lab efficiency.

The use of the Method Development System also allows time for other tasks in the lab. This would experimental sequence would normally require repetitive changing and equilibrating columns during the process. With this system the chromatographer can preload mobile phase composition and location and preload column descriptions and locations. The system will then use the chromatographer's method flow to chose the correct column and mobile phase combination needed for each experiment. The system will operate unattended while the user works on other tasks.