Automated Method Development Using UV and MS Detection

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Abstract

Traditionally HPLC UV methods have been developed on UV only systems. This is can be time consuming because it requires an individual injection of a standard of each component in the mixture. This injection series must be repeated on each column with each experimental variable change.

By adding the specificity of single quadrapole Mass Spectroscopy detection to the Method Development Process, we can reduce the number of injections to 1 injection per variable.

Introduction

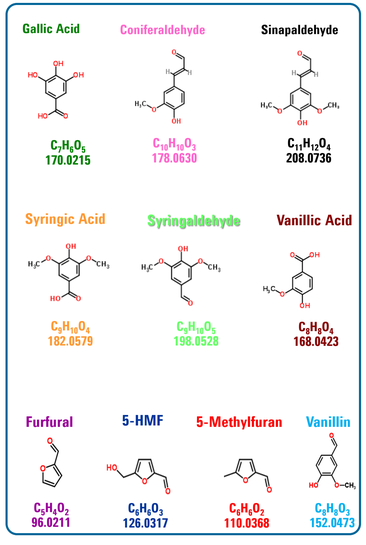
There are many congeners found in real bourbon as a result of the fermentation, distillation, and the aging process. These congeners make each bourbon unique. Traditionally present in true bourbon are phenols, furans and antioxidants such as: gallic acid, vanillic acid, syringic acid, ellagic acid, syringaldehyde, 5-(hydroxymethyl) furfural, and 2-furaldehyde. The presence and concentration of these congeners is the key in determining if bourbon is genuine. GC and GC/MS are often used for analysis of these compounds, however, these analytes require derivatization for GC analysis.

Here we look at a group of non-volatile phenolic compounds and furans, using reverse phase LC with ESI single Quadrapole MS detection The goal is to develop a HPLC method to be used with UV only detection in a Quality Assurance Laboratory. We also want to determine whether Methanol or Acetonitrile is a better organic mobile phase.

If we were to do the method development with UV only detection, we would need to run 10 individual standards of each of the congeners and the mix. Doing method development under these conditions would require 11 runs per experimentental variable. We will use 6 columns which is 66 runs and 2 mobile phases which will increase the total runs to 112. The run time using our scouting gradient conditions will be 15.4 hours (942 minutes) even with the time saving advantages of sub 2 micron particle columns. This does not include data analysis and review time to confirm peak identification.

However, with the addition of MS detection we will be able to run the same experimental conditions in less than 90 minutes. We will be able to accurately track peak elution order and peak identification as selectivity and solvent strength change. This will be done automatically using the Extracted Ion Chromatogram of each of the congeners in the Mass Hunter Qualitative Data Analysis Method. The file will contain the UV trace and the overlay of all the EICs allowing for instant data review.

Non-Volatile Congeners Structures



Experimental

HPLC Conditions

Agilent 1290 Infinity HPLC series Binary Pump, Well Plate Sampler, Thermostatic Column Compartment, DAD

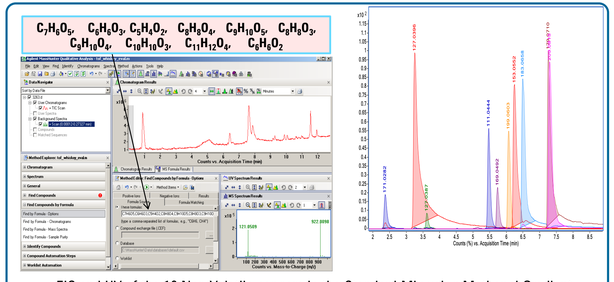
Column: Zorbax Eclipse Plus, C18 2.1 x 50mm, 1.8 µm
Phenyl-Hexyl
SB-C8
Cyano
AQ
Extend C18

Column temperature:	35°C
Injection volume:	0.5 µL
Autosampler temp:	ambient °C
Needle wash:	10 s Flush Port (25:25:50) (H_2O:IPA:MeOH)
DAD-UV	280 nm
Mobile phase:	A = 0.1% Formic Acid in Water B1 = 0.1% Formic Acid in Methanol B2= 0.1% Formic Acid in Acetonitrile
Flow rate:	0.3 mL/min
Gradient:	Time (min) %B 0.0 5 0.7 95
Stop time:	5.0 min.
Post time:	2.0min.
Overall run time	7.0minutes (incl. re-equilibration)

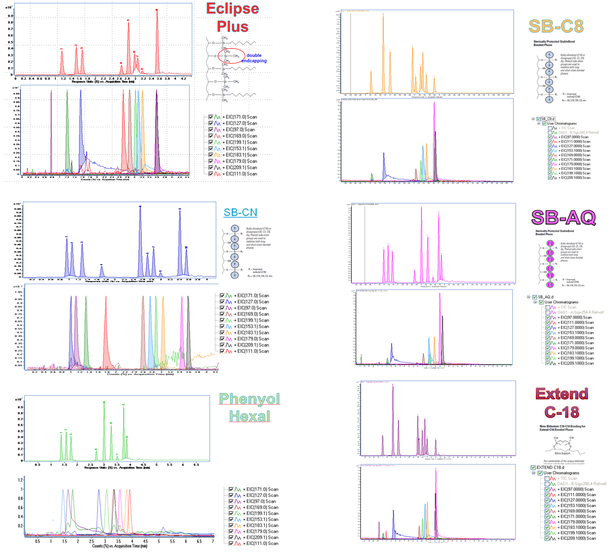
MS: Agilent 6130B Single Quadropole Mass Spectrometer

MS Parameters	
Ion Mode	ESI,
PositiveMass Range	90-1200 m/z
Scan Rate	2 Hz
Source Parameters	
Drying gas (Nitrogen)	10 L/min
Drying gas temperature	300 °C
Nebulizer gas (Nitrogen)	40 PSI
Scan Source Parameters	
Capillary Voltage	4000 V
Fragmentor	145 V

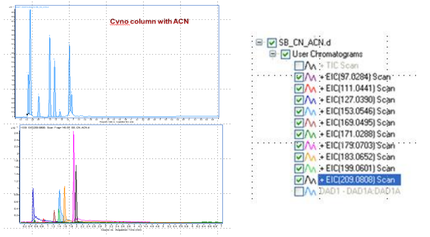
Results and Discussion



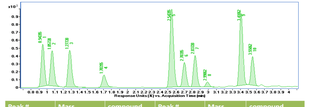
EIC and UV of the 10 Non Volatile congers in the Standard Mix using Methanol Gradient. Data Analysis done with a single Data Analysis Method in Mass Hunter Software



EIC and UV of the 10 Non Volatile congers in the Standard Mix comparing the Methanol versus Acetonitrile as the strong solvent keeping the same K*



Final UV Chromatogram with baseline separation on all 10 congener peaks developed in less than 90 minutes with positive identification of all 10 peaks



Peak #	Mass	compound	Peak #	Mass	compound
1	127.0390		6	153.0546	vanillin
2	97.0284	5-HMF	7	199.0601	Syringic acid
3	171.0288	furfural	8	183.0652	Gallic acid
4	111.0441	5-methylfuran	9	179.0703	syringaldehyde
5	169.0495	vanillic acid	10	209.0808	coniferaldehyde
					sinapaldehyde

Conclusions

Single Quadrople detection added to the Method Development process can greatly decrease the time and solvent needed to create a new analytical method to be used for UV only detection. With a single qualitative method in Mass Hunter we were able to simple scroll through the data and decide that the UV separation with the Cyano column with a Simple Methanol/ Water and Formic Acid gradient was an effective UV method. The use of volitle buffers is not only Mass Spec friendly, but will allow the user less wear and maintance issues than the traditional salt buffers on the HPLC system .