Abstract

Traditionally, HPLC UV methods have been developed on UV systems, thus in this case we can test measuring bi-aromatic substances in the sample with an HPLC method. Eclipse 145 V - detecting added to the Method Development process can significantly increase the total number of injections to 112. The run time using our scouting conditions will be 15.4 hours (942 minutes) even with the gradient conditions. The goal is to develop a HPLC method to be used with UV and MS detection to the Method Development process, we can reduce the number of injections to 10, decreasing the run time of each experimental variable.

Non-Volatile Congeners Structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>Formula</th>
<th>Mass Hunter Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic Acid</td>
<td>C_6H_6O_5</td>
<td>170.0255</td>
</tr>
<tr>
<td>Coniferaldehyde</td>
<td>C_8H_8O_3</td>
<td>168.0423</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>C_9H_8O_3</td>
<td>198.0528</td>
</tr>
<tr>
<td>Vanillin</td>
<td>C_9H_8O_4</td>
<td>152.0473</td>
</tr>
<tr>
<td>Furfural</td>
<td>C_5H_4O</td>
<td>100.0688</td>
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<tr>
<td>Sinapic Acid</td>
<td>C_9H_8O_4</td>
<td>168.0423</td>
</tr>
<tr>
<td>Coniferyl alcohol</td>
<td>C_9H_8O_5</td>
<td>198.0528</td>
</tr>
</tbody>
</table>

Introduction

There are many congeners found in real bourbon as a result of the fermentation, distillation, and aging process. These congeners include non-volatile compounds and the presence of these congeners is key in determining if bourbon is genuine. GC and HPLC-MS are often used for analysis of these congeners; however, these analyses require derivatization for GC analysis.

Here we look at a group of non-volatile phenolic compounds and flavans, using reverse phase C-18 with ESI quadrapole MS detection. To identify each congener detected in a Quality Assurance Laboratory. We also want to determine detection Methanol or Acetone in a final Roger’s mobile phase.

If we were to use the method developed with UV only detection. We would want to test 10 different standards of each of the congeners and the mix. Using method development under these conditions would require 11 runs per experimental variable. We will use 2 columns which is 60 mm 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) with the final run time of about 3 minutes retention per congener. This does not include data analysis and review time in the lab.

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 necessary and retain strength changes. This will be done using a scouting gradient series of 0-90% methanol in 12 minutes.

Experimental

HPLC Conditions

Agilent 1200 infinity HPLC series Binary Pump, Volt-Plate Sampler, Thermostatic Column Compartment; DAD

Column: Zorbax Eclipse Plus, C18 2.1 x 53mm, 1.8 µm

Flow Rate: 0.2 mL/min

Gradient: 0.5-5 % Methanol (V/v)


DAD UV:

Column temperature: 35°C

Injection volume: 10 µl

Autosampler: temperature 4°C

Flow rate: 1 ml/min

Mass Hunter Qualitative Data Analysis done with a single Data Analysis Method in Mass Hunter Software.

EIC and UV of the 10 Non Volatile congeners in the Standard Mix using Methanol Gradient.

Post Gradient:

Flow rate:

Needle wash:

10 s Flush Port

Ambient

10 s

Ion Mode:

Positive

Scan Rate:

0.2 Hz

Scan Range:

350-550

Nebulizer:

40 PSI

Source Parameters:

Drying gas (Nitrogen) 18 L/min

Drying gas temperature 350°C

B2 = 0.1% Formic Acid in Water

B1 = 5% Formic Acid in Methanol

Data Analysis done with a single Data Analysis Method in Mass Hunter Software.

Conclusions

Single Quadrapole detection added to the Method Development process can greatly reduce the time and solvent needed to create a new analytical method. The results and data reviewed will be used in the Agilent Technologies proven Method. The file will contain the UV traces and the overlay of all the EICs allowing for instant data review.

Results and Discussion

EIC and UV of the 10 Non Volatile congeners in the Standard Mix comparing the Methanol versus Acetone as the strong solvent keeping the same EIC.

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

Poster #