

Identification and quantitation of degradation products in corn stover hydrolyzate for the biofuels industry

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1. Overview

An HPLC-MS/MS method was developed for the profiling and quantitation of corn stover hydrolyzate samples for known phenolic fermentation inhibitors.

2. Introduction

Biomass, such as corn stover can be converted to ethanol to replace fossil fuels, but enzymatic hydrolysis limits the amount produced. Corn stover hydrolyzate contains a variety of degradation products, including aliphatic and aromatic acids, aromatic aldehydes and phenols, which can

inhibit microbial processes and reduce the bioconversion of cellulosic material to ethanol. Currently, 9 phenolic acids are monitored by HPLC-UV, but many more aliphatic acids are relevant, but do not contain chromophores.

3. Methods

Sample preparation: A standard mixture of nine phenolic compounds and a corn stover hydrolyzate sample were provided by a biofuels company. Optimized MRM transitions were found for most compounds and SIM conditions were used when no stable product ion was observed. 500 µL of hydrolyzate was extracted two times with 4 mLs of ethyl acetate and combined, then dried under nitrogen. One hydrolyzate sample was spiked with 5

µL of the phenolic standard mix prior to extraction, and one spiked post extraction. The samples were brought up in 200 µL of mobile phase A and vortexed.

Data analysis: The method of standard addition was used for quantitation since a blank sample was unavailable. The calibration curve was not weighted and not forced through zero.

Table1 UHPLC conditions used for the analysis

System:	Nexera-LCMS8040
MP A:	0.1% FA in Water
MP B:	0.1% FA in Acetonitrile
Gradient:	0%B isocratic for 1.0 min, gradient to 20% B until 7.0 min, gradient to 85%B at 9.0 min, then reequilibration at 0%B until 12.0 min
Column:	Synergi® Hydro-RP 2.5 µm 100 Å, 100 × 2.0 mm
Flow Rate	0.4 mL / min. 3400psi.
Column Temp:	40°C
Injection Vol:	10 µL

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Quantitation of known fermentation inhibitors

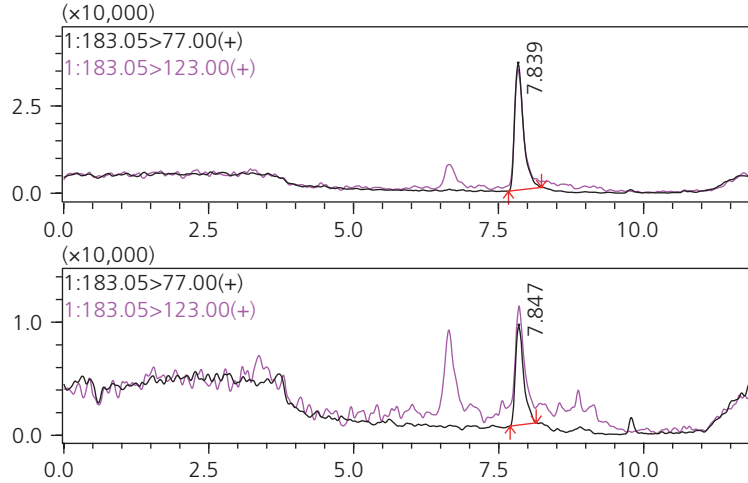


Fig. 1 Example chromatograms for a standard (top) and corn stover hydrolyzate (bottom) for the two MS-MS transitions of syringaldehyde

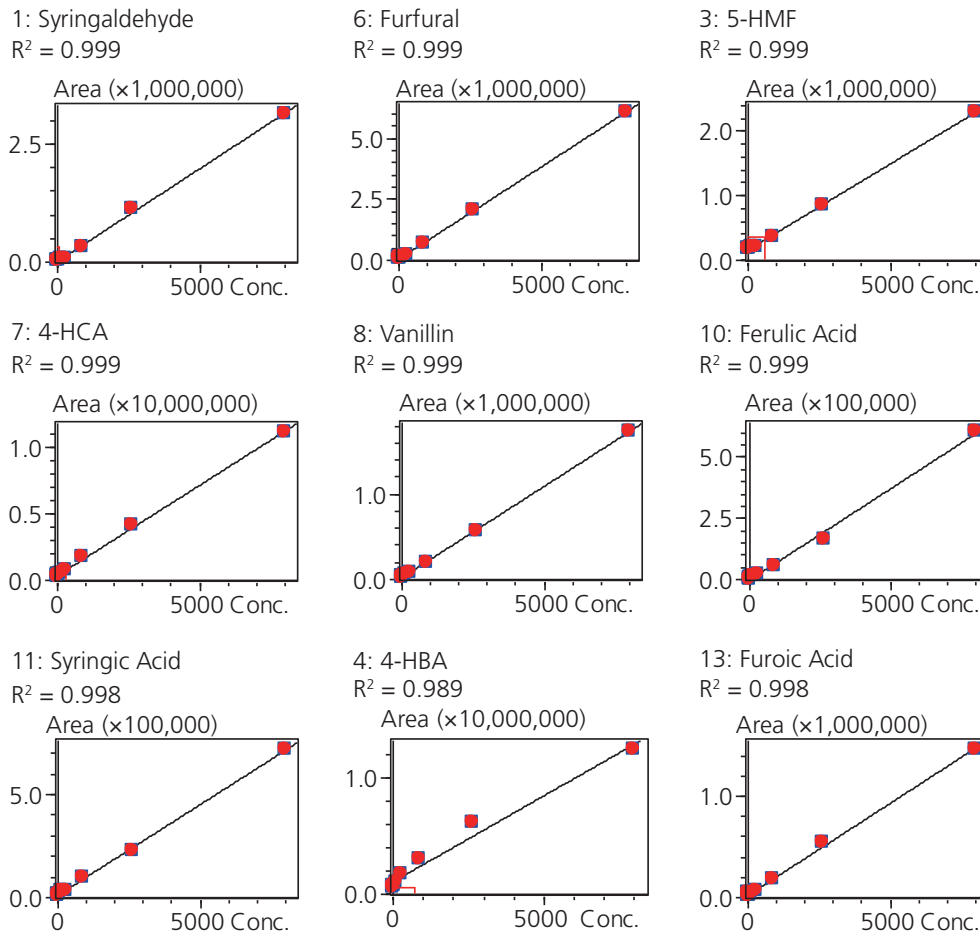


Fig. 2 Matrix matched standard curves for the nine phenolic fermentation inhibitors from 10.9 to 8000 ng/mL. No data points were omitted.

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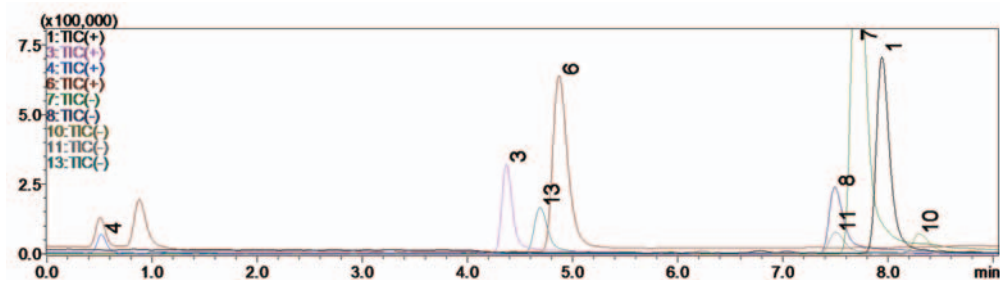
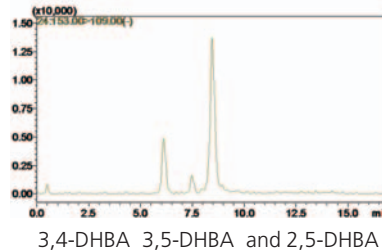
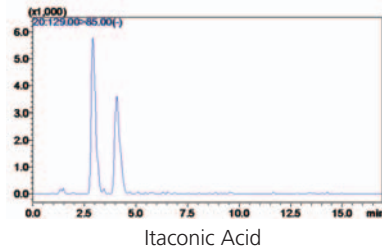
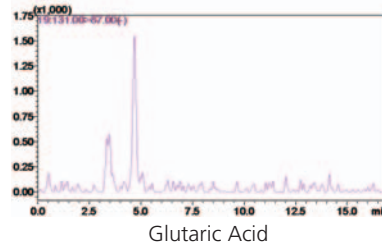
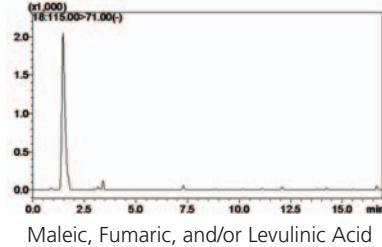
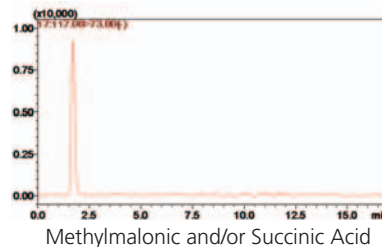
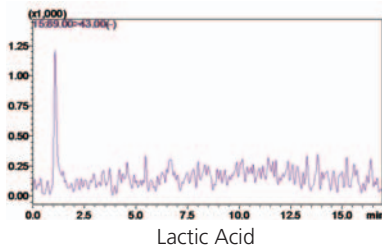


Fig. 3 Overlaid TICs for each of the nine fermentation inhibitors at the high standard curve point

Table 2 Concentration of known fermentation inhibitors in corn stover hydrolyzate

#	Name	ng /mL
1	Syringaldehyde	14.4
6	Furfural	27.2
3	5-HMF	242.0
4	4-Hydroxycoumaric Acid	106.0
8	Vanillin	43.2
10	Ferulic Acid	15.2
11	Syringic Acid	54.4
4	4-Hydroxy butyric Acid	291.2
13	Furoic Acid	49.6

Identification of previously unmonitored fermentation inhibitors



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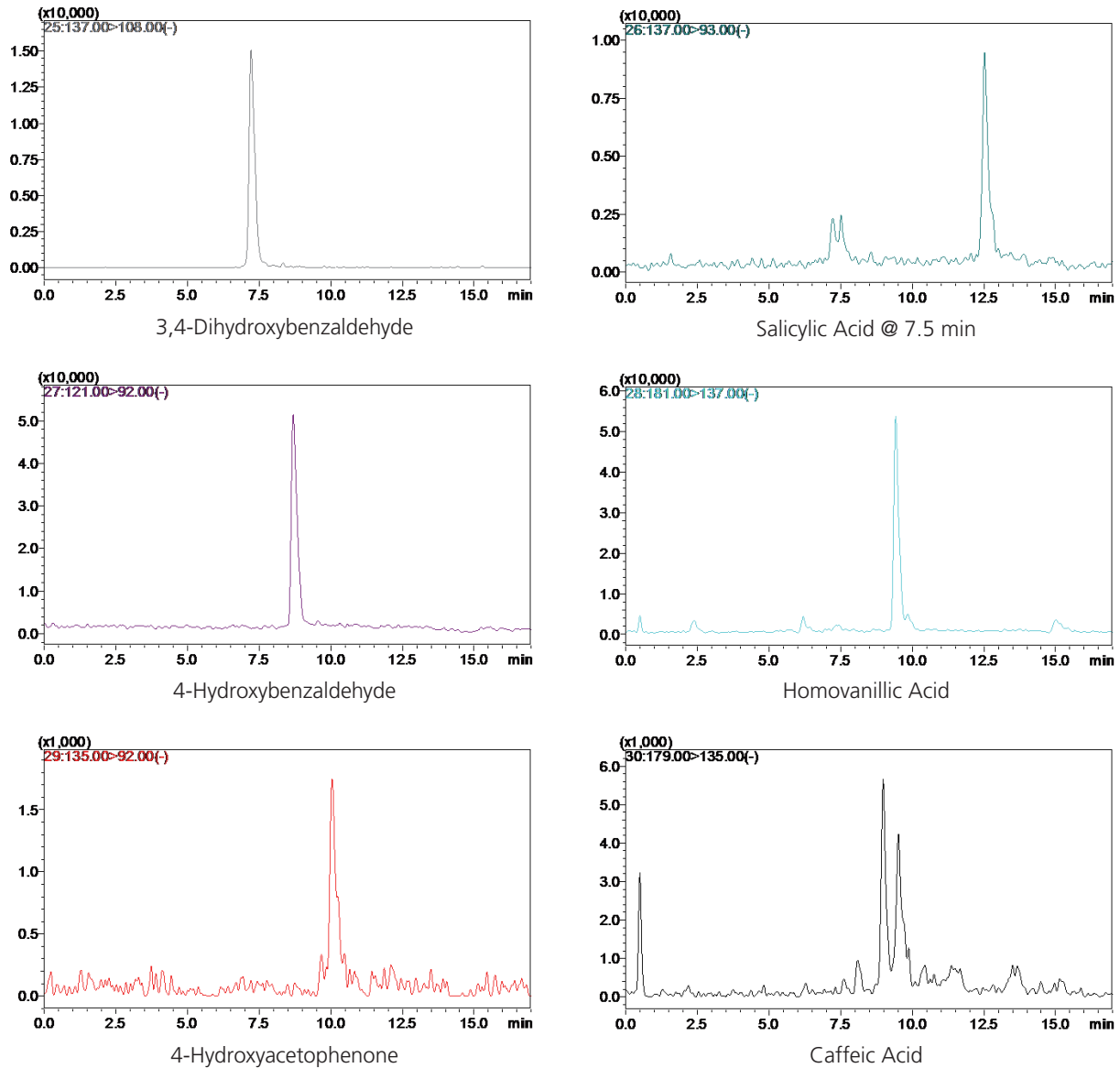


Fig. 4 Twelve additional chromatograms of fermentation inhibitors expected to be found in corn stover hydrolyzate. Relative retention times and MS-MS data were used for tentative identification.

4. Conclusions

It is possible to quantitatively analyze corn stover hydrolyzate over 3 orders of magnitude, from low ng/mL to low µg/mL without internal standards. A decision to terminate fermentation can be made when inhibitors reach a predetermined concentration. Compounds were identified and differentiated from isobars by spiking in phenolic standards and observing peak area increases. The

compounds were further verified by comparison of product ion scans of the standard and the unspiked corn stover hydrolyzate sample. 14 compounds were tentatively identified by acquiring expected transitions and comparing retention times to the published data¹. Analytical standards of the proposed compounds should be purchased and analyzed for confirmation.

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5. References

1 Sharma, L. N. Identification and Quantitation of Potential Fermentation Inhibitors in Biomass Pretreatment Hydrolyzate Using HPLC in Combination with UV Detection and Tandem MS. Ph.D. dissertation, Baylor University, Waco, TX 2009