Article source technical application "Liquid-Liquid Extraction (LLE) vs. Novum™ Simplified Liquid Extraction (SLE) Case Study: Analysis of Monomeric Phenolic Fermentation Inhibitors in Dilute-Acid Plant Hydrolysate"

Pretreatment of lignocellulosic biomass is a prerequisite in order to maximize glucose release from cellulose for subsequent fermentation or chemical conversions. A leading pretreatment involves dilute acid (e.g., 0.3 – 2% sulfuric acid) at elevated temperatures (140 to 200 °C) with different holding times and biomass/acid loadings1. A drawback of this procedure is the partial degradation of lignin, a polyphenolic macromolecule, to smaller phenolics that are released together with the sugars into the so-called hydrolysate. These phenolic compounds are toxic to microorganisms and are potential fermentation inhibitors 2-5.

Analyzing this phenolic profile is important when optimizing pretreatment or detoxification methods aiming to minimize inhibitor levels or to engineer more resistant microorganisms. In a widely employed sample preparation and cleanup step, the phenolic compounds undergo a liquid-liquid extraction using an organic solvent (e.g., ethyl acetate) and are then analyzed via GC/MS6.

While effective, the liquid-liquid extraction can be time-consuming, requiring 4 separate extractions and multiple centrifugation steps. In an effort to reduce time and sample handling steps, a simplified liquid extraction (SLE) using Novum SLE was employed, saving a significant amount of time and reducing the amount of required pipetting and vial handling steps.

Materials and Methods

Lignocellulosic biomass was prepared using two different methods, a traditional liquid-liquid extraction and simplified liquid extraction (SLE) using a Novum SLE MAX 96-well plate (part no. 8E-S138-5GA). Prior to each extraction method, the samples were pretreated as follows:

Pretreatment

A dilute-acid pretreatment hydrolysate (1.5% sulfuric acid, 190 °C for 1 minute, 25% biomass loading) from Miscanthus X giganteus was filtered using a PES (polyethersulfone) 0.45 µm filter and was spiked with 20 µg/mL iso-propylphenol as an internal standard (IS). The pretreated samples were then extracted by either liquid-liquid extraction or SLE (below).

Liquid-Liquid Extraction Procedure

1 mL aliquots of the spiked hydrolysate from the pretreatment step were extracted 4x each with 0.5 mL of ethyl acetate. After each extraction, a centrifugation step (1500 g for 1 minute) was applied for phase separation. The combined extracts of each sample were dried over sodium sulfate6 (samples A).

Simplified Liquid Extraction (SLE) Procedure

400 µL aliquots of the spiked hydrolysate from the pretreatment step were loaded into the

wells of a Novum SLE MAX 96-well plate. The sample was allowed to soak into the sorbent for 5 minutes and then the ethyl acetate was applied to elute the analytes of interest. Three different volumes were applied to determine the most effective elution volume. Either 1x 0.4 mL (samples B), 1x 0.8 mL (samples C), or 2x 0.4 mL (samples D) of ethyl acetate was applied using a short pulse of vacuum. No further drying of ethyl acetate was performed after the elution. GC/MS Conditions

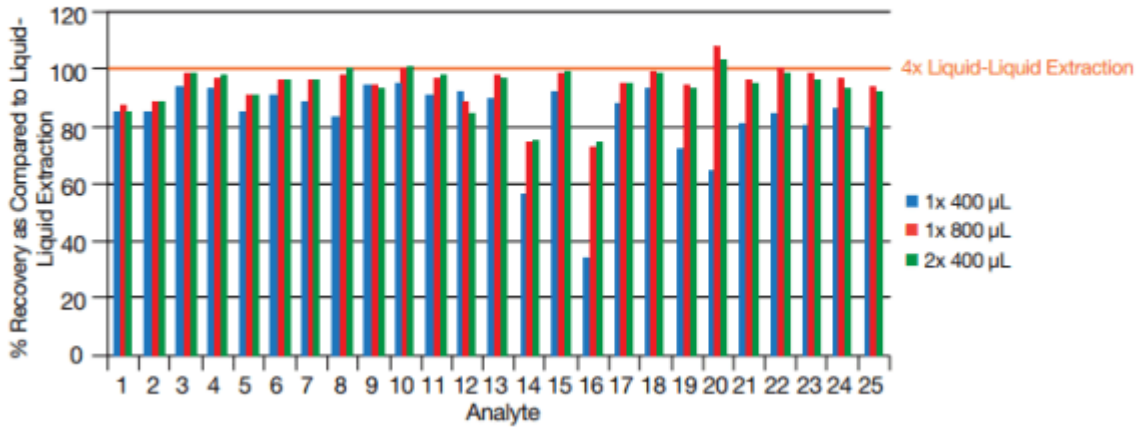
Prior to GC analysis, 100 µL aliquots of the extracts (samples A – D) were incubated with 50 µL BSTFA at 70 °C for 30 minutes and then analyzed by GC/MS6. All extractions were performed in triplicate and replicates (1-3) were run in the following order: samples A1 – D1, then A2 – D2, then A3 – D3 to avoid any bias because of potential changes in sample composition of unprocessed samples due to the long run time (67 minutes). 1 µL was injected in splitless mode onto a 5-ms capillary GC column (30 m x 0.25 mm x 0.25 µm).

An Agilent® 7890A gas chromatograph coupled to an Agilent 5975C single quadrupole mass spectrometer was used for analysis with the following settings: Injector and transfer line temperature 280 °C, carrier gas: helium at 1 mL/min, temperature program: 75 °C for 3 min isocratic, 5 °C/min to 150 °C, 0.5 °C/min to 160 °C, 2 °C/min to 190 °C, 5 °C/min to 240 °C, 70 °C/min to 325 °C, 3 min isocratic. Ions were detected in full scan mode m/z 35-500.

Results and Discussion

Figure 1 shows the relative recoveries of 25 selected phenolic compounds from hydrolysate eluted from the Novum SLE MAX 96-well plate using the 3 elution procedures (0.4 mL, 0.8 mL and 2x 0.4 mL) compared to the values obtained by the conventional liquid-liquid extraction (=100% on the representative chart).

Figure 1. Relative recovery (%) after extraction using Novum SLE under various extraction solvent volumes as compared to a liquid-liquid extraction



Analyte Key:

1	4-hydroxybenzaldehyde
2	vanillin
3	acetovanillone
4	4-hydroxybenzoic acid
5	syringaldehyde
6	vanillic acid
7	homovanillic acid
8	3,4-dihydroxybenzoic acid
9	4-hydroxy-3-methoxy-cinnamaldehyde
10	syringic acid
11	trans-p-coumaric acid
12	sinapaldehyde

Analyte Key *cont'd*:

13	trans-ferulic acid
14	2-guaiacylacetaldehyde
15	guaiacylacetone
16	2-syringacetaldehyde
17	3-guaiacylpropanol
18	2-hydroxy-1-guaiacylpropanone
19	3-guaiacylacetol
20	3-syringylacetol
21	1-guaiacylacetol
22	3-(4-hydroxyphenyl)acetol
23	1-syringylacetol
24	2-hydroxy-1-guaiacylethanone
25	2-hydroxy-1-syringylethanone

Overall the Novum SLE extraction produced recoveries that were comparable to the traditional liquid-liquid extraction procedure in a fraction of the time (a five-fold decrease in the amount of sample preparation time). The 0.4 mL ethyl acetate elution resulted in a general lower recovery of the analytes compared to the 0.8 mL and 2x 0.4 mL volume extractions. Exceptions were 4-hydroxy-3-methoxy-cinnamaldehyde (Figure 1, analyte 9)

with almost identical recovery levels observed (95% and 93%, respectively) for all elution volumes and sinapaldehyde (Figure 1, analyte 12) with a higher recovery for the 0.4 mL extraction volume (93 %) compared to the other extraction volumes applied (89 % and 85 %, respectively). However, a 0.4 mL extraction volume was generally deemed to be suboptimal. Increasing the elution volume to 0.8 mL improved the recovery with most recoveries reaching >90%. Slightly lower yields were observed for 4-hydroxybenzaldehyde (87%) (Figure 1, analyte 1) and vanillin (89%) (Figure 1, analyte 2). Only the homologs 2-guaiacylacetaldehyde (Figure 1, analyte 14) and 2-syringacetaldehyde (Figure 1, analyte 16) exhibited lower recovery levels of 75-76% and 73-75%, respectively. Applying the 0.8 mL elution volume in two steps (2x 0.4 mL) did not improve the recovery as compared to a single aliquot of 0.8 mL. TN-0082 Page 2 of 4 APPLICATIONS

Conclusion

When extracting 0.4 mL of a dilute-acid plant hydrolysate using a Novum™ SLE MAX 96-well plate, a 0.8 mL elution volume of ethyl acetate resulted in high recoveries of >90% for most phenolic analytes. No further drying of the ethyl acetate phase was necessary to ensure successful derivatization for subsequent GC/MS analysis. Compared to the conventional liquid-liquid extraction, we estimate an up to a five-fold decrease in sample preparation time.

Furthermore, the Novum SLE extraction also greatly simplifies sample preparation by reducing the amount of required pipetting and vial handling steps. Therefore, sample preparation using the Novum SLE resulted in a phenolic inhibitor profile that was very similar and comparable to the conventional liquid-liquid extraction but at a fraction of the

time and labor.

References

1. *Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, Schoen P, Lukas J, Olthof B, Worley M, Sexton D, Dudgeon D (2011) Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. Technical Report NREL/TP-5100-47764. United States National Renewable Energy Laboratory, US Department of Energy. May 2011*
2. *Jönsson LJ, Palmqvist E, Nilvebrant N-O, Hahn-Hägerdal B (1998) Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus Trametes versicolor. Appl Microbiol Biotechnol 49:691-697*
3. *Luo C, Brink DL, Blanch HW (2002) Identification of potential fermentation inhibitors in conversion of hybrid poplar hydrolyzate to ethanol. Biomass Bioenergy 22:125-138*
4. *Larsson S, Reimann A, Nilvebrant N-O, Jönsson LJ (1999) Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. App Biochem Biotechnol 77-79:91- 103*
5. *Buchert J, Niemelä K, Puls J, Poutanen K (1990) Improvement in the fermentability of steamed hemicellulose hydrolysate by ion exclusion. Process Biochem Int 176-180*
6. *Mitchell VD, Taylor CM, Bauer S (2014) Comprehensive analysis of monomeric phenolics in dilute acid plant hydrolysates. Bioenerg Res 7:654-669*

Share with friends and coworkers:

- Click to email this to a friend (Opens in new window)
- Click to share on Twitter (Opens in new window)
- Click to share on Facebook (Opens in new window)
- Click to share on Pinterest (Opens in new window)
- Click to share on LinkedIn (Opens in new window)
- Click to share on Tumblr (Opens in new window)
- Click to share on Reddit (Opens in new window)