

Organic Acids in Silage

Application Note

Food and Environmental

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Introduction

In addition to other factors, the concentration of the three fermentation acids lactic acid, acetic acid, and butyric acid is a criterion for the quality of silages. HPLC is the choice for this analysis, since volatile and nonvolatile acids can be determined together without prior derivatization. This application note shows the analysis of several specimens of silages (grass, whole plant, and corn) using an Agilent Hi-Plex H column.



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Materials and Methods

The extraction of the acids is done according to EN 13037 (soil improvers and growing media determination of pH) by adding 1.25 L of water to 250 mL of silage and agitating for one hour. The sample was pretreated by filtration through a 0.45 µm membrane.

Conditions

Column	Agilent Hi-Plex H, 7.7 x 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.005 M H ₂ SO ₄
Gradient	Isocratic
Flow rate	0.7 mL/min
Injection volume	20 µL
Sample concentration	Glucose 50 – 1500 mg/L Succinic acid 50 – 125 mg/L Lactic acid 750 – 1000 mg/L Acetic acid 200 – 450 mg/L Ethanol 80 – 700 mg/L
Temperature	60 °C
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	RI (55 °C)

Results and Discussion

Figure 1 shows the analysis of grass silage, which has undergone a homofermentative process leading mostly to lactic acid and a small amount of ethanol. An example for a heterofermentative process is shown in Figure 2. Here the silage of corn yielded not only lactic acid, but also acetic acid and ethanol. Figure 3 shows the analysis of whole plant silage, which has undergone an untypical process, leaving a large amount of free sugars.

Conclusion

Samples of silage from different crops were successfully separated by HPLC with an Agilent Hi-Plex H column.

Hi-Plex H is the column of choice for the analysis of organic acids in complex matrices, using dilute mineral acid as eluent. Hi-Plex columns are packed with sulfonated resin, giving a fundamental improvement in performance. They contain monodisperse sulfonated packing to overcome the problems of low efficiencies and high backpressures encountered with soft gels.

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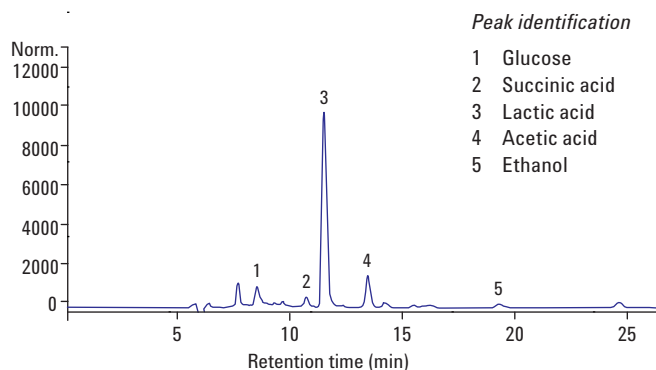


Figure 1. Analysis of grass silage using an Agilent Hi-Plex H column.

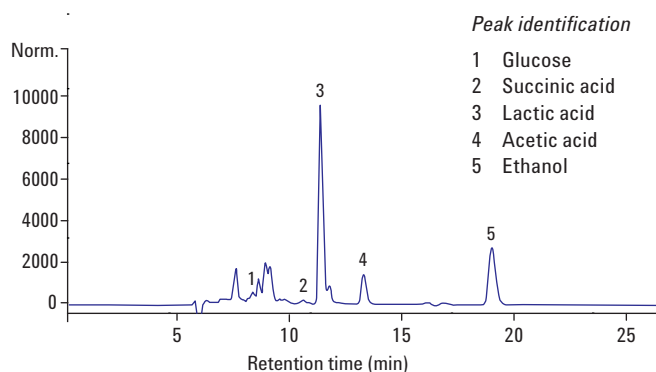


Figure 2. Separation of corn silage using an Agilent Hi-Plex H column.

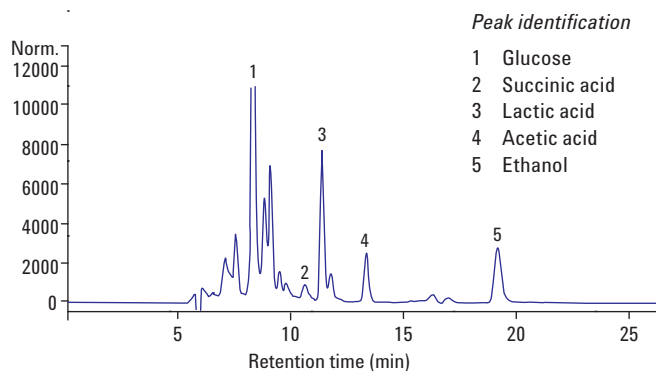


Figure 3. Analysis of whole plant silage using an Agilent Hi-Plex H column.

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© Agilent Technologies, Inc., 2011
Published in USA, June 30, 2011
SI-1945



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