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Profiling of Complex Polysaccharides in Honey Samples by HILIC-HRMS

Nithin Sajeev[1], Trivikram Reddy Gundala, PhD[2], Dipankar Malakar, PhD[1], Rajesh Babu Dandamudi, PhD[2], and Bryan Tackett, PhD[3]
[1]SCIEX Lab, Hitech Defence and Aerospace Park Industrial Area, Mahadeva Kodigehalli, Hobli, Jala Taluka, Begaluru 562149, India
[2]India PhenoLogix Lab, Phenomenex Inda, Hitech Defence and Aerospace Park Industrial Area, Mahadeva Kodigehalli, Hobli, Jala Taluka, Bengaluru 562149, India
[3]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Natural honey consists of both simple and complex sugars. In addition, honey is also known to contain more than 180 minor components. This makes the analysis of honey challenging. Honey is also prone to adulteration by adding simple sugars. The content of higher polysaccharides can therefore be used to establish the authenticity of honey. Sugars, in general, are chromatographically analyzed in Hydrophilic Interaction Liquid Chromatography (HILIC) mode. High Resolution Mass Spectrometry (HRMS) can be used as a powerful mass detection tool to identity the exact masses of the complex sugars. Therefore, combining the HILIC chromatography with HRMS can be beneficial for profiling the complex polysaccharides, especially in the absence of standards. In this technical note, we have developed a HILIC method on a Venusil 3 µm HILIC column for the profiling of these polysaccharides. The SCIEX® X500R QTOF System was used for HRMS analysis.

Sample Preparation

Honey samples were obtained from commercial stores. One gram of the honey sample was weighed and diluted using 4 mL of water. The solution was mixed well and centrifuged for 10 min. The supernatant was filtered and taken for LC-HRMS analysis.

LC Conditions

Column: Venusil 3 µm HILIC
Dimensions: 100 x 2.1 mm
Part No.: VH931002-0
Mobile Phase: A: 2 mM Ammonium Formate in Water
B: Acetonitrile

Gradient:

Time (min)	%B
0	70
2.5	70
5	45
7.5	45
7.55	70
9	70

Flow Rate: 0.4 mL/min
Injection Volume: 5 µL
Temperature: 50 °C
LC System: SCIEX ExionLC™
Detection: HRMS
Detector: SCIEX X500R QTOF

HRMS Conditions

Ion Source: TurboIonSpray®
Scan Type: TOFMS
Source Temperature: 500 °C
Polarity: Negative
CUR: 30 psi
GS1: 50 psi
GS2: 45 psi
Spray Voltage: -4500 V
CAD: 6
Declustering Potential: -80 V
TOF Stop Mass: 2400 Da
Accumulation Time: 1 sec

Results and Discussion

The chromatographic parameters were optimized to get better resolution and peak shape. The method was successfully verified for the separation of polysaccharides from DP 13 to DP 23 (**Figures 1** and **2**) within a short run time of a 6-minute gradient. Polysaccharides were identified based on their corresponding precursor m/z. In this study, peak area of different dilutions of a low abundant polysaccharide form (DP 23) was considered to determine linearity, reproducibility, and accuracy of the measurement. To assess the reproducibility of the method, the different dilutions of the sample were injected in five replicates and the %RSD and % CV were maintained below or equal to 2.17 and 5.2, respectively (**Table 1**). The linearity was verified by preparing a calibration plot using different dilutions of the sample and found to be very close to 1.0 (**Figure 3** and **Figure 4**). Moreover, accuracy range found between 89.9 and 112.4. The optimized method was then used for the relative quantitation of DP23 present in three different commercially available samples. The three different honey samples found to contain polysaccharides in varied quantities (**Figure 5**).

Figure 1. Separation of Polysaccharides in a Honey Sample Using a Venusil 3 µm HILIC Column.

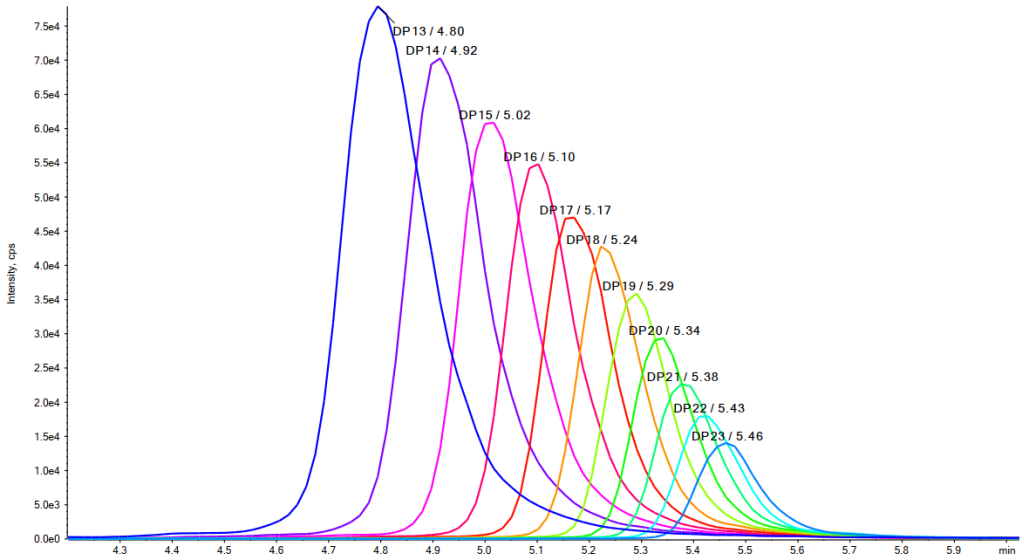


Figure 2. Overlay of Three Replicates of Honey Samples.

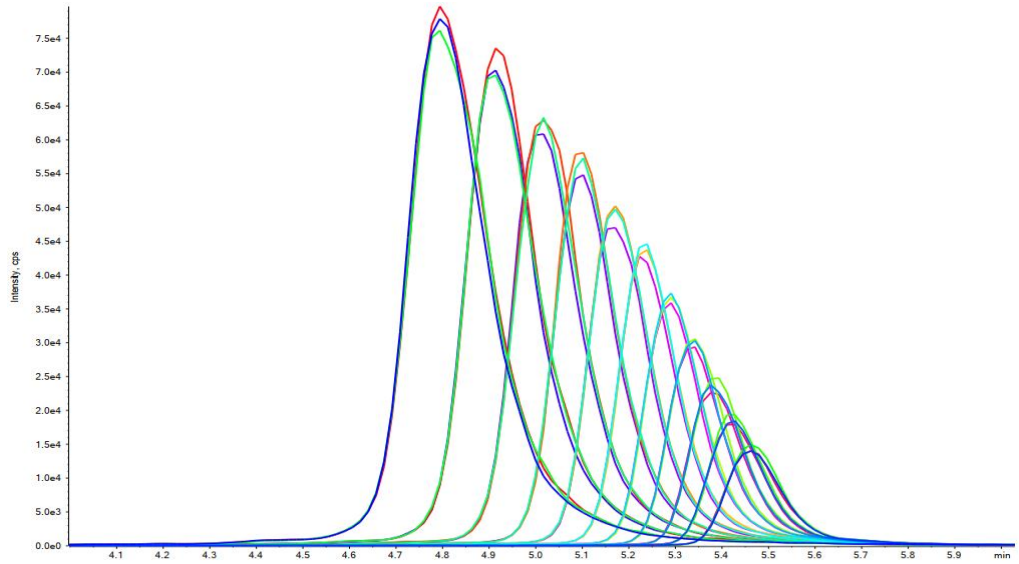


Figure 3. Calibration Curve of DP 23 Standard.

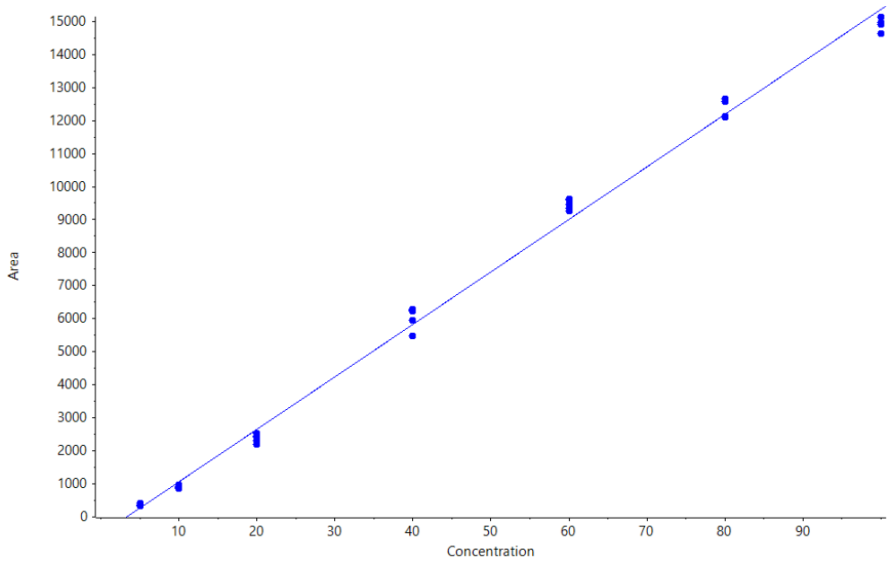


Table 1. Linearity, Accuracy, and Reproducibility Within Various Dilutions of Sample (n=5).

Expected Concentration	Number of Values	Mean Calculated Concentration	Average Accuracy	Standard Deviation	% CV
5	5	5.62E+00	112.4	0.15	2.8
10	5	8.99E+00	89.9	0.32	3.5
20	5	1.83E+01	91.6	0.8	4.4
40	5	4.13E+01	103.3	2.17	5.2
60	5	6.28E+01	104.7	0.94	1.5
80	5	8.08E+01	101	1.7	2.1
100	5	9.71E+01	97.1	1.14	1.2
Regression Equation: y = 158.92521x + -525.68072; r2 = 0.99609; Weighting: 1/x					

Figure 4. Linearity Levels of DP 23 Standard Concentrations.

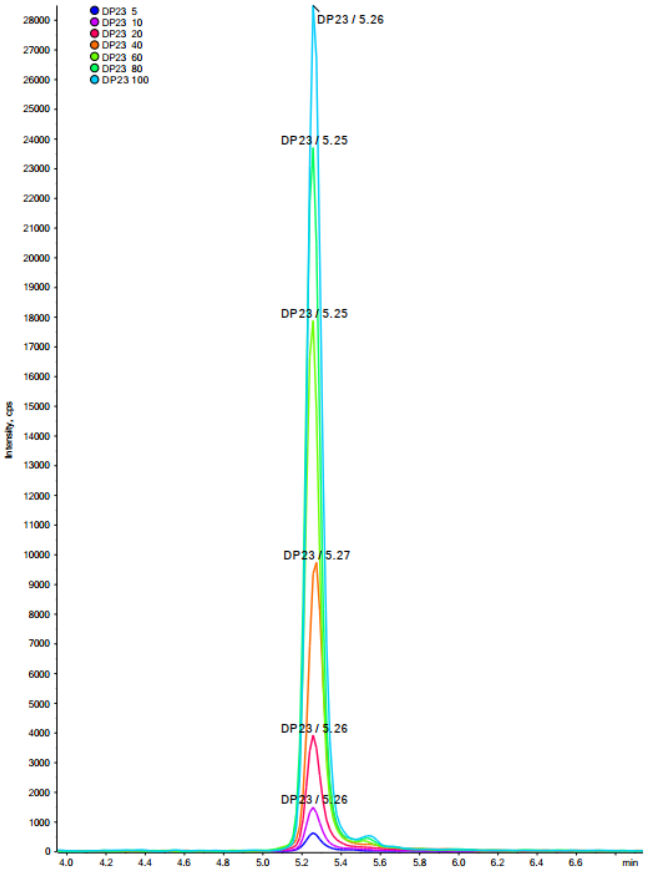
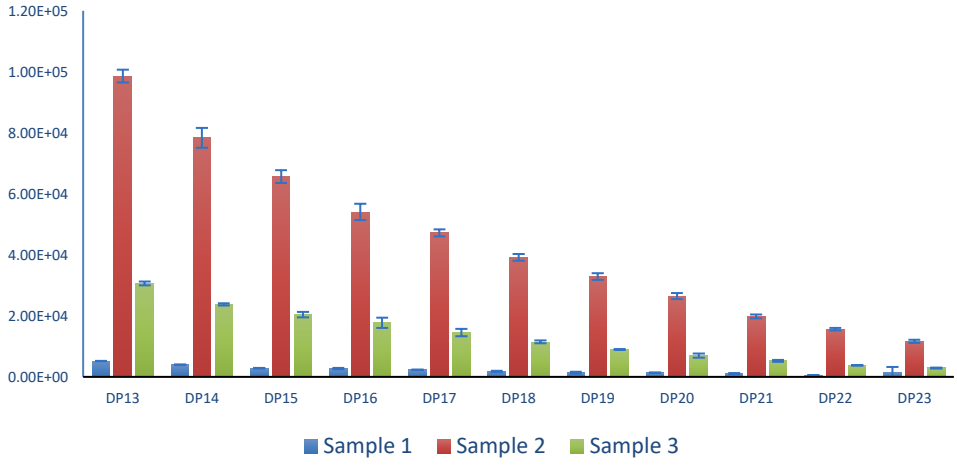


Figure 5. Variations in Different Polysaccharide Levels Among the Three Different Honey Samples (Mean ± SD, n=3).



Conclusions

A simple and robust method for profiling of complex polysaccharides have been developed using HILIC-HRMS technique. The method is a simple gradient using Ammonium Acetate buffer and Acetonitrile. The entire run was completed in just 6 minutes. The Venusil 3 µm HILIC column with amide chemistry proved to have effective selectivity in resolving the closely related polysaccharides. The three commercial samples showed variations with respect to their polysaccharide content. This method could be useful for establishing the authenticity, geographical origin, and adulteration (if any) of honey samples.

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t: +61 (0)2-9428-6444
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Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
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