Honey Compositional Analysis by HPLC

Application Note

Food Testing & Agriculture

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Introduction

Honeys vary in their composition from type to type. The purpose of analyzing honey is to find out which sugars are present and in what percentage.

High Performance Liquid Chromatography has been used for the analysis of honey composition. In this application note, a method of analyzing this type of sample is described.

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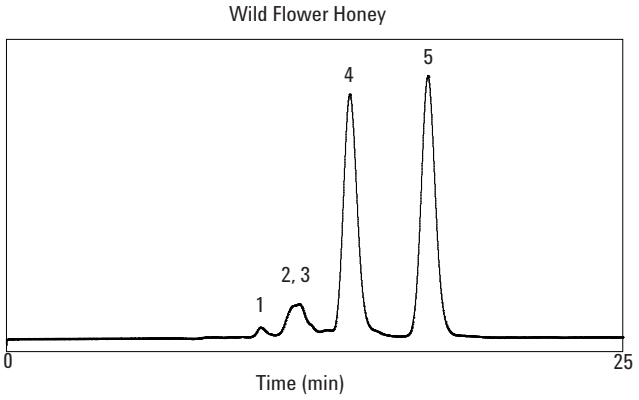
Sample Preparation

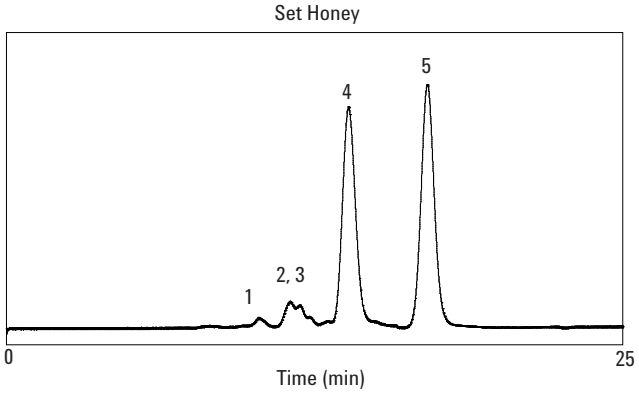
Samples of four different varieties of honey were collected and stored in glass jars (25 mL). They were then weighed out and dissolved in water in order to obtain 20 mg/mL solutions of each.

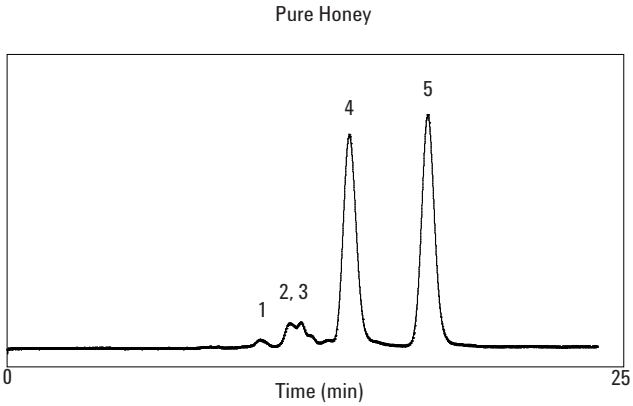
HPLC Conditions

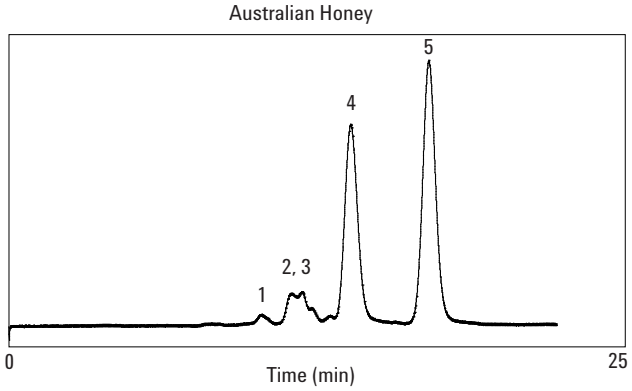
20 µL of each sample was injected onto an Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm column at 85 °C with a flow rate of 0.6 mL/min. Pure water was used as eluent, and detection carried out on an Agilent RI detector. All injections were made in duplicate.

Results and Discussion









Compounds: 1) Melezitose, 2) Sucrose, 3) Maltose, 4) Glucose, 5) Fructose

Individual injections of standard solutions were made to confirm the identities of the components of each honey sample, and the peak areas recorded to calculate the percentage of each sugar in the 4 different honeys (Table 1).

Table 1. Composition of different types of honey revealed by HPLC

Experimental Honey Composition	Wild Flower Honey	Set Honey	Pure Honey	Australian Honey
Melezitose (%)	0.5	1.0	0.6	1.1
Sucrose + maltose (%)	3.9	3.8	3.8	5.4
Glucose (%)	30.3	33.9	32.8	31.0
Fructose (%)	41.5	47.8	45.7	50.4
Water (%)	23.8	13.5	17.1	12.1

A "typical composition" for honey is shown in Table 2 for comparison with the experimental results.

Comparing published data with the percentage compositions acquired through chromatography, some differences are

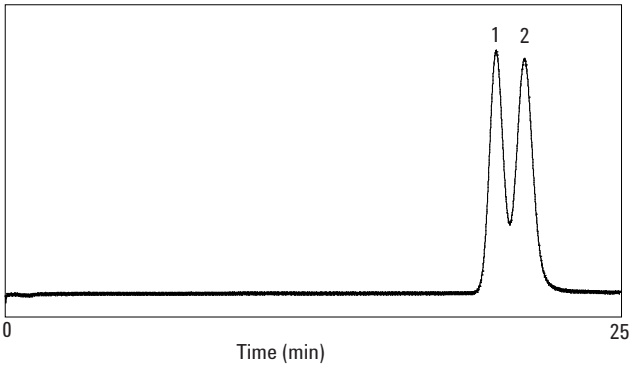
apparent. Percentages of the disaccharides maltose and sucrose, plus the trisaccharide melezitose, did not correspond in the two analyses. These three sugars combined reached a maximum of 6.5% experimentally but 10% theoretically. The laboratory analysis also revealed a high amount of fructose (41.5 – 50.4%), which was 38.5% in published data. This indicates that the sugar content of honey must vary significantly from one type to another.

From a chromatography point of view, the Hi-Plex Ca column was unable to separate sucrose and maltose, which have therefore been quantified together in this report. A few attempts at separating these two sugars were made; the flow rate was decreased (from 0.6 mL/min down to 0.4 mL/min) and the temperature increased (from 85 up to 90 °C), with little success.

Therefore, if separation of sucrose and maltose is required, particularly from a quantification point of view, an alternative column type would need to be considered. For example, the Agilent Hi-Plex Pb offers alternative selectivity to the Hi-Plex Ca, with increased retention and selectivity for certain disaccharides and is able to give partial separation of sucrose and maltose, as follows.

Table 2. Honey composition from published data

Theoretical Honey Composition (from research)	Honey
Melezitose and maltose (%)	9.0
Sucrose (%)	1.0
Glucose (%)	31.0
Fructose (%)	38.5
Water (%)	17.0
Ash (%)	0.17
Other (vitamin, minerals, antioxidants)	3.0



Agilent Hi-Plex Pb, 7.7 × 300 mm. Mobile Phase: Water at 0.6 mL/min, 70 °C Compounds: 1) Sucrose, 2) Maltose

Conclusion

This report demonstrates how an Agilent Hi-Plex Ca column can be used to quantify the levels of the sugars that make up honey, and shows that there is significant variation from type to type.

It also highlights the benefits of changing column phase (in this case, from Hi-Plex Ca to Hi-Plex Pb) in gaining a separation of closely eluting analytes.

These data represent typical results.

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