Simple, Robust and High-throughput Analysis of Organic acids in Honey Using LC-MS/MS

Durvesh Sawant [1], William Lipps[2], Nitin Shukla [1], Anjali Mishra[1], Samruddha Chavan[1], Nitish Suryawanshi[1], Dr. Pratap Rasam[1], Dr Jitendra Kelkar[1] .
[1]Shimadzu India Pvt Ltd, 5th Floor, ABR Emerald, Plot No. D-8, Street no. 16, MIDC, Andheri East, Mumbai 400093, Maharashtra, India; [2]Shimadzu Scientific Instruments, Inc.102 Riverwood Drive, Columbia, MD 21046, U.S.A.

1. Introduction

Honey (Figure. 1) being a natural energy booster packed with healthy carbohydrates for quick, clean energy. It also has medicinal properties where it soothes coughs & throats. Acts as a natural, thick demulcent to calm irritation. Rich in antioxidants, contains bioactive plant compounds that support overall health. Supports skin healing, natural antibacterial properties help soothe minor burns and wounds. Being an universal remedy product, it makes the honey manufactures compulsive to maintain the quality of the honey. One of the parameter that decides the quality of honey is Organic acids. The determination of organic acids in honey is critical for assessing product quality, freshness, authenticity, and botanical and geographical origin. Major organic acids, including gluconic, citric, and malic acids, contribute significantly to the sensory characteristics, stability, and overall quality of honey. Their compositional profiles also provide valuable information for the detection of spoilage and adulteration with sugar syrups. Certain organic acids serve as important indicators of honey quality and processing conditions. Organic acid profiling has also proven effective for distinguishing nectar honey from honeydew honey, detecting sugar adulteration, and authenticating floral origin. Elevated levels of acetic acid, for example, are commonly associated with yeast fermentation and inadequate storage practices. Furthermore, formic acid is widely applied in apiculture for the control of Varroa mites, necessitating residue monitoring to ensure consumer safety. Pyruvic acid has likewise been reported as a useful marker for the differentiation of unifloral honey varieties. Various analytical techniques have been explored for quantifying these parameters for the quality and authenticity of honey samples[1]. In response to increasingly stringent regulatory requirements for honey quality and authenticity testing, this study aimed to develop a sensitive and reliable analytical method for the trace-level determination of organic acids in honey. The proposed method utilizes a simple liquid–liquid extraction (LLE) procedure coupled with LC–MS/MS analysis and achieves a limit of quantification (LOQ) of 1.20 ppm and LOD of 0.025 ppm.
Multiple Reaction Monitoring (MRM) based LC-MS/MS techniques are widely used on triple quadrupole platforms for quantitation. Here, UFMS (Ultra-Fast Mass Spectrometry) technology and the Corespray™ technology of LCMS-8045RX (Shimadzu Corporation, Japan) (Fig.2) has been used for rapid, reliable and sensitive quantitation of organic acids in complex matrix like honey. Shimadzu Shim-pack GIST Phenyl Hexyl column was used to retain organic acids with reversed phase chromatography.

Figure. 1 Raw Honey Sample

2. Materials and method

The organic acid standards were procured from Sigma Aldrich. Further all individual standards stock solution were prepared in water. Further mixture of all stock solution was prepared in water. This stock was used to prepare the calibration levels. LCMS-8045RX coupled with Nexera™ series by Shimadzu, set a new benchmark in UHPLC coupled with triple quadrupole. Nexera™ series with its Automated support functions utilizing analytical intelligence technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher throughput and maximum reliability. LCMS-8045RX with an unsurpassed sensitivity (UFsensitivity),ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). CoreSpray™ Heated ESI for improved nebulization with ion focus unit to route target ions effectively and reduced noise. This combination ensures highest quality of data, with very high degree of reliability. Auto MRM optimization feature was used for optimization for MRM transition (Table 2.)

Figure 2. Shimadzu LCMS-8045RX triple quadrupole with Nexera X3 liquid chromatography

2-1 Instrument details

All organic acids were analysed using ultra high-performance chromatography (UHPLC) Nexera *X3* coupled with LCMS-8045RX triple quadrupole system (Shimadzu Corporation Japan). The details of analytical conditions ref Table. 1

Table.1 UHPLC conditions (Nexera X3 system)

Parameter	Value
Column	Shim-pack GIST Phenyl Hexyl
Mobile Phase	A : 2.5 mM Ammonium carbonate in water B : 100% Acetonitrile
Flow method	Gradient
Flow rate	1 mL/min
Injection Vol	40 µL
Column Temp	40°C
Needle wash	Water: Methanol (1:1 v/v)

MS conditions (LCMS-8045RX) Ionization: Corespray ESI, Positive MRM mode

Parameter	Value
Nebulizing gas flow	3 L/min
DL Temperature	250 ºC
Interface Temperature	400 ºC
Heat Block Temperature	400 ºC
Drying gas flow	3 L/min

Table 2: MRM details

Compound name	Precursor ion (m/z)	Product ion (m/z)	CE (Volts)
Formic acid	45.20	45.20	2.0
Acetic acid	59.10	58.95	11.0
Citric Acid	190.90	110.95	13.0
Pyruvic acid	87.20	42.95	2.0

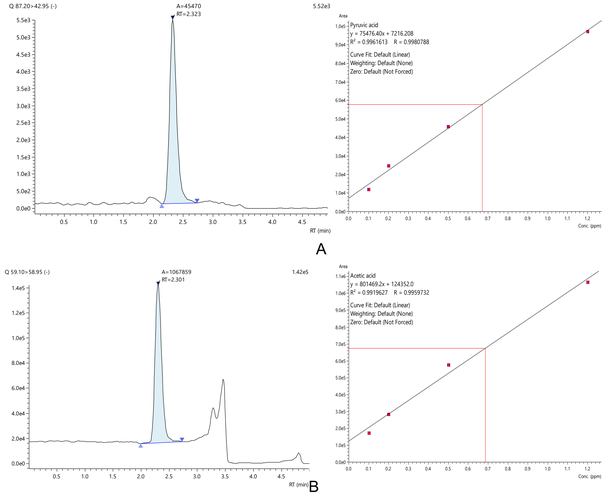

Figure 3: A- Pyruvic acid, B- Acetic acid,

2.2 Sample extraction

Commercially available honey sample was used for analysis. The sample preparation method is as given below.

Take 0.5 g of pre spiked sample into 50 mL tube. Add 12 mL of MS grade water, vortex for 5 min.

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Centrifuge at 5000 rpm for 5 min.

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Filter the aliquot solution using nylon 0.22 micron filter. Transfer to HPLC vial and inject.
Note: Final dilution factor 25

3. Results

The quantitation was performed using matrix matched standard. Linearity studies were carried out using external calibration method. The calibration levels were prepared and injected in MRM mode. Linearity range were as follows: Acetic acid and Pyruvic acid (0.1 to 1.2 ppm), Citric acid (0.025 to 0.35 ppm), Formic acid (1 to 5 ppm). An accuracy of ± 20% for the injected calibration standards was observed for all compounds. The representative chromatogram, calibration curve are shown in Figure 3.
Recovery study was performed at 5 ppm level for Acetic acid and Pyruvic acid, 1.25 ppm for Citric acid and at 10 ppm for Formic acid. Recovery results were found to be between ± 10% (Table:3).

Table 3: Sample results

Compound	Formic acid	Acetic acid	Citric Acid	Pyruvic acid
Spiked conc. mg/kg	10	5	1.25	5
Observed conc. mg/kg	40.67(30 mg/kg incurred)	5.23	1.29	5
% recovery	102	105	103.2	100

4. Conclusion

A cost-effective, green chemistry analytical method was developed featuring a simplified two-step sample preparation protocol. By minimizing chemical and reagent consumption, the approach aligns with sustainable practices while consistently delivering accuracy and precision within acceptable regulatory limits.

5. References

1. Mato, I., Huidobro, J.F., Simal-Lozano, J., Sancho, M.T., 2006. Analytical Methods for the Determination of Organic Acids in Honey. Crit. Rev. Anal. Chem. 36, 3–11. doi:10.1080/10408340500451957