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Short Communication

Analysis of Honeys by Ultra Performance Liquid Chromatography Coupled to Mass Spectrometry

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ABOUT THE STUDY

Honey has been extensively studied due to the increased interest in both food and medicinal consumption. There is a concern with the correct identification and quantification of the present compounds, as well as the verification of the chromatographic profile of the honeys of different blooms. The development of techniques capable of separating complex mixtures, identifying and quantifying the components is a useful tool that can be well applied in the analysis of honey samples. The Ultra-High Performance Liquid Chromatography (UHPLC) coupled to the mass spectrometer is a powerful tool for the analysis of complex samples. This is because it is more selective and has high sensitivity allowing the obtaining of qualitative data, enabling the quick determination of the chromatographic profile of the samples. In addition to allowing the annotation of the substances that may constitute the sample by providing the obtainment of m/z ratios, as well as ions/molecular formulas and fragmentation profiles.

The chemical substances present in honey can be biologically active with important therapeutic properties. Since the compound is a natural product of botanical origin and has particular properties due to the geographic location and the species of bees involved in the synthesis [1]. Among the types of honey produced, the monofloral ones have peculiar characteristics due to their botanical origin, the pollen that determines the variety directly contributes to the biochemistry and organoleptic properties of the product [2]. The determination and characterization of the chemical profile of honey is an important tool for the valorization of this product. Honey consists of several classes of organic compounds such as phenolic acids, flavonoids, terpenes, vitamins and others that have biological activity. Thus, it is necessary to elucidate the chemical profile of honey for the identification or investigation of active substances for use in the formulation of medicines and products. Thus, the choice of a method capable of elucidating components and molecular formulas by protonization or deprotonization processes, such as UHPLC-MS, is extremely important for the investigation of honey components. Instrumental analyzes of analytical chemistry have developed rapidly and new technologies have facilitated the study of complex mixtures. Among these techniques, UHPLC coupled to MS stands out, which facilitates the separation of compounds, allowing the observation of the chromatographic profile and, in the sequence, they are identified by Mass Spectrometry (MS) based on spectroscopic patterns. UHPLC has gained notoriety in recent years due to greater efficiency in the compound separation process, in addition to better peak resolution and lower solvent consumption when compared to conventional HPLC [3]. This makes this equipment and the method essential tools for determining the chromatographic profile and the investigative approach in the analysis of substances of pharmacological and biotechnological interest in honeys of different flowerings [4]. Thus, the objective was to adapt a UHPLC-MS methodology, operated in negative polarity that has sensitivity for detecting compounds in honeys.

Reagents

Ultrapure water (MilliQ), Methanol (Merck), Ethyl acetate (Vetec), NaCl (Sigma Aldrich), Na_2SO_4 (Biotec), Formic acid (Vetec), Acetonitrile (Merck), Standards (Sigma Aldrich).

Honey extraction

Due to the high viscosity of the honey sample, it is necessary to previously heat the samples in a water bath at 45°C, and then prepare a 2% (w/v) NaCl solution in which 1 g of NaCl is dissolved in 50 mL of ultrapure water. Next, 5 mL of this saline solution is added to 5 g of honey. This material was subjected to vortex agitation for 10 seconds and then three extractions of 5 mL each were performed with ethyl acetate. After this liquid-

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liquid extraction, Na_2SO_4 is added to the organic phase as a drying agent, and then the solution obtained is stirred and filtered through cotton into a previously weighed scintillation vial. The ready solution is submitted to speedvac equipment, obtaining a dry concentrated honey sample.

Sample preparation

The dried honey sample, previously obtained, was dissolved in a methanol/water solution (3:2, v/v), obtaining a final concentration of 5 ug/uL. After this dilution, the sample is submitted to ultrasound for 10 minutes, centrifuged at 10,000 RPM for 15 minutes. Afterwards, it is stored at room temperature for about 24 hours.

Preparation of solutions for analysis

The previously prepared honey samples, dissolved in a 3:2 (v/v) methanol/water solution, were solubilized according to the dry sample mass obtained after extraction; adding the volume of methanol/water solution 3:2 (v/v), necessary to obtain them at a concentration of 5 ug/uL. The sample solutions were transferred to UHPLC automatic injection vials. To control the extraction process, a solution of 1000 uL of methanol/water was prepared. As reference substances, the following standards were used: gallic acid, caffeine, quercetin and rutin. Standard solutions were diluted in a 3:2 (v/v) methanol/water solution, according to their respective molar masses, obtaining solutions at a concentration of 5 ug/uL.

Analysis method UHPLC-MS/MS

The analysis was performed using a UHPLC system, connected to a Nexera SIL-30AC autosampler and a Nexera SPD-M20A DAD detector, supervised by a Nexera 20A CBM (Shimadzu, Japan). Chromatographic separation was performed using a Shimpack XR-ODSIII, C18, 2.2 um, 80A, 2.0 × 150 mm column (Shimadzu, Japan). The chromatograph is coupled to a mass spectrometer with time-of-flight detection model, maXis-ETD ESI-QqTOF model (Bruker, Germany). Elution was performed using as mobile phase (A): water acidified with 0.1% formic acid and (B): acetonitrile acidified with 0.1%formic acid. Linear gradient from 5% to 95% B in 15 minutes was used. Between injections of 5 uL of each sample, the column was reconditioned with 95% B for 3 minutes and 5% B for 6 minutes. The spectrometer operated using ESI as the ion source type; negative ionization mode; nebulizer gas: 3.0 Bar; drying gas flow 8 L/min; temperature: 200°C; spectra were recorded in scan mode from 100 to 1500 m/z.

Current trends reveal research on polyphenolic profiling and quantification of honey compounds with mass-hyphenated UHPLC techniques [3,5]. The use of UHPLC as a chromatographic technique allows obtaining the chemical profile of complex samples, such as honey, quickly and efficiently, in addition to being highly selective and sensitive when compared to other chromatographic techniques; allowing the simultaneous detection of different compounds and with high resolution. In addition, when coupled to the mass spectrum, it allows the annotation of the compounds that constitute the sample [6,7].

CONCLUSION

The method used in the present work allowed obtaining the chromatographic profiles of honey samples from different botanical origins. Through the technique used, UHPLC-MS, it was possible to identify similarities and differences in the chemical profiles; and make notes and predictions of the substances that can compose each honey. This, through the analysis of the retention time, molecular formula and fragmentation profiles obtained. The use of standards also allows inferring about the possible presence of the substance, as well as ensuring the suitability of the system for analyzing the class of compounds expected to be present in the analyzed samples. In addition, the analysis of the blank of the process, as well as the blank of the eluent system; allows the exclusion of possible interferences and guarantees that the substances eluted in the respective retention times shown in the chromatograms, are compounds present in the samples. This study demonstrates that the UHPLC-MS technique, operated in negative polarity, has high sensitivity for detection of phenolic components. This allowed the detection of a wide range of compounds, enabling the characterization of the chemical profile of complex samples, such as honeys of different flowerings.

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