Mini Review
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Qualitative study of Armenian honey (stevia) by mass-spectrometer

Abstract

The presence of Stevia extracted from Armenian leafs provedby modern instrumental method. For the qualitative analysis of aqueous solution obtained from the extract of Armenian honey leaf (Stevia), Sciex's Triple Quad™ 4500 Quadruple Mass Spectrometry has been used, for a sensitive and selective structural detection of Steviol.

Keywords: armenian honey leaf, stevia, steviol glycoside, sweetener, mass spectrometry method of analysis, MS/MS method

Volume 4 Issue 1 - 2020

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Received: December 25, 2019 | **Published:** February 4, 2020

Introduction

Nowadays there are dozens of herbs growing in Armenia that contain glycosides and are considered natural sweeteners. They are guaranteed by scientists for use in food.[1] In this regard, gallicidal glandular derivatives from stevia are particularly interesting. Stevioids, reboid A, B, C, D and E, dulcosideA andsteviololiosis. Their sweetness exceeds 100 to 400 times the sucrose. Today, there are about 200 species of quails grown primarily in Paraguay, Brazil and South-East Asia, as well as in the United States, Israel, and Ukraine. Climatic conditions in Armenia allow growing a variety of stevia, which is called Armenian honey. This plant is quite fruit-bearing and 20-25 leaves can be harvested from one shrub. It is known that Stevie glycoside, stevioside and rebaudioside A are responsible for the sweet taste of leaves. It is also known that steviol glycosides structure are very similar to each other and have no big differences.[2] The structural similarity creates difficulties in chromatographic separation.[3] In addition, there are certain difficulties in determining possible differences in ultraviolet and infrared spectroscopy.

A number of methods are used for the study of the natural sugar and artificial sweeteners. The most effective methods are the highly effective liquid cryoprophy (DIMEX) Dionex Thermo Scientific Corona™ Charged, a CAD detector, which provides sensitivity for low concentrations, with great reproducibility and accuracy. It was also decided that steviol glycosides can be charged aerosol and identified by UV detection based on using towers Acclaim Trinity P_1.[4] The authors find that the method of charged aerosols is flexible and has advantages over the other PMSC methods. It also allows checking the sweetness of the sweetener. This work is presented in the Askeran region of Nagorno-Karabakh grown leafy water extract findings made for clarification of the composition. The aim of using mass spectrometer detection method (MS/MS) to have more sensitive and selective results. The MS methods nowadays are widely used for scientific and applied purposes, particularly in medical, pharmaceutical and other studies.[5,6] We have investigated preliminary an extract of Armenian honey leaf (stevia) by Mass spectral method.[1] The purpose of the presented work is to use the above-mentioned quick and sensitive method for qualitative study of Armenian honey.

Materials and methods

Stevia extract was supplied by the National Polytechnic University of Armenia supplier. The study of stevia's presence in aqueous solution was carried out using Scitex Triple Quad™ 4500 Quadruple Mass Spectrometer. The sample dilution was carried out using HPLC Grade Methanol and Deionizer water supplied by Carl Roth, Germany. Formic Acid (98% ACS grade) Carl Roth (Germany). The instrument and chemicals were supplied by Dramatist Laboratories, Armenia.

The extracted sample was diluted to have a concentration in the ng/mL range, (1:1) Methanol:Formic Acid (0.1% in water v/v) was used for dilution. The diluted sample was introduced to the MS by a syringe pump with a flow rate of 7µl/min.

Results and Discussion

The tuning of MS parameters was conducted in positive and negative ionization mode, however the intensity of the signal in the positive mode was significantly lower than the negative mode, therefore the further optimization carried out in the negative mode. The Q_1 full scan spectra from 100 to 1000D_a gave predominant singly charged de protonated precursor [M-H]-ions at m/z of 803.4D_a (Figure 1).

In the product ion mass spectrum of 803.4D_a, the most abundant and consistent ions were observed at m/z 641.3D_a (Figure 2) which can be defined as the Stevioside [$C_{38}H_{60}O_{18}$] minus the lower cycle [$C_{32}H_{49}O_{13}$].As fragments intensity in the Triple quad mass spectrometry depends mainly on the collision energy (CE), thus when increasing the CE we observed high intensity at 479.2D_a (Figure 3), which can be defined as the Stevioside [$C_{38}H_{60}O_{18}$] minus the upper cycles [$C_{26}H_{39}O_8$].

To attain a better impact on the spectral response, an optimum potential of -4500V was kept, which gave a consistent and stable signal. Fine-tuning of the Declustering Potential (DP)-60V, Entrance Potential (EP)-10V, Collision Energy for the highest intensity fragment (641.4D_a) (CE)-20V and the Collision Cell Exit Potential (CXP) -12V was conducted to produce consistent and stable responses, and the dwell time of 200ms was adequate.

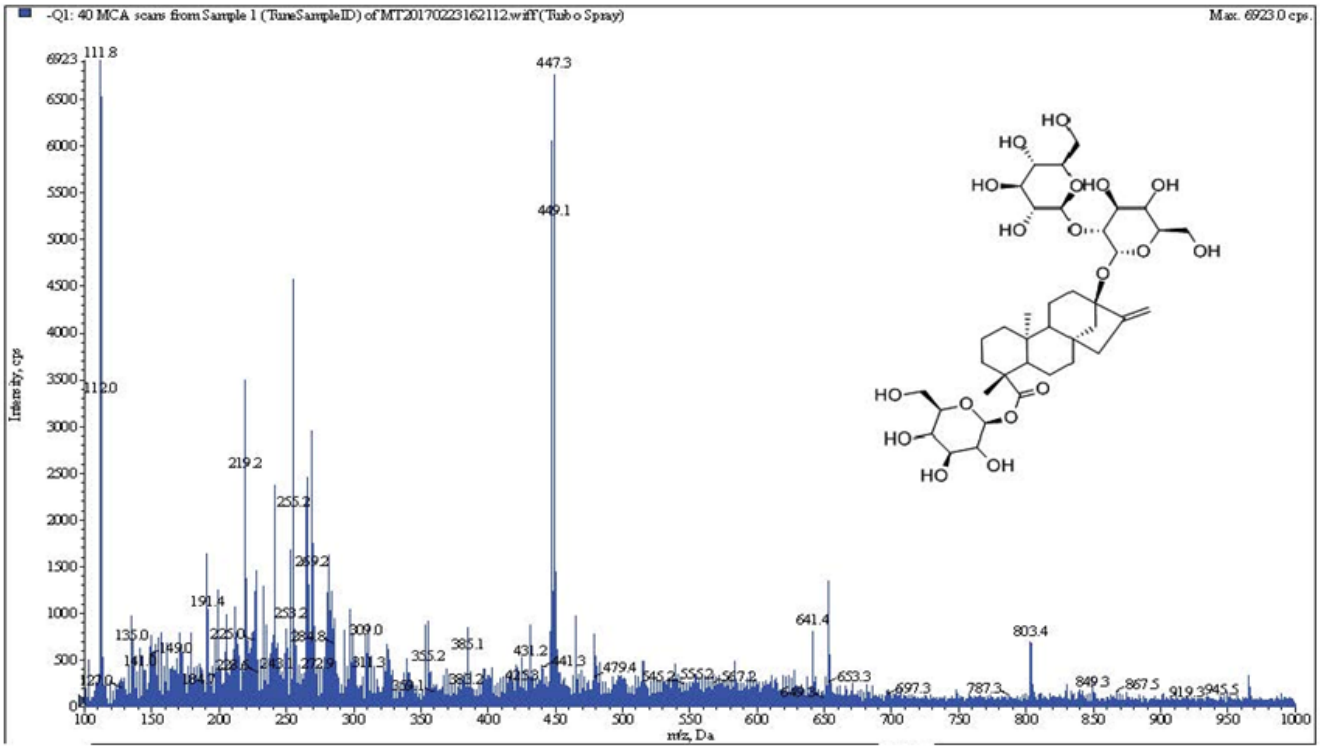

Figure 1 Q1 scan spectra.

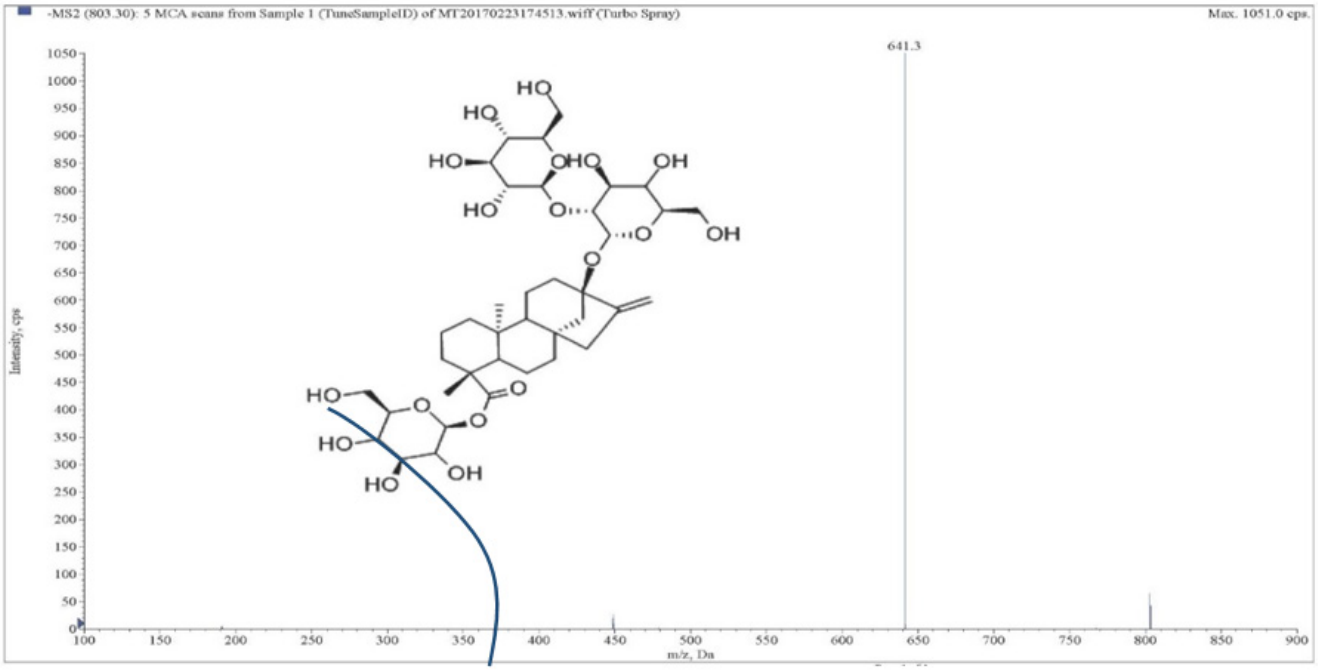

Figure 2 Q3 scan (product ion) spectra A.

Citation: Torosyan GH, Armoudjian Y, Ayriyants MA, et al. Qualitative study of Armenian honey (stevia) by mass-spectrometer. *MOJ Biorg Org Chem.* 2020;4(1):18–20. DOI: 10.15406/mojboc.2020.04.00105

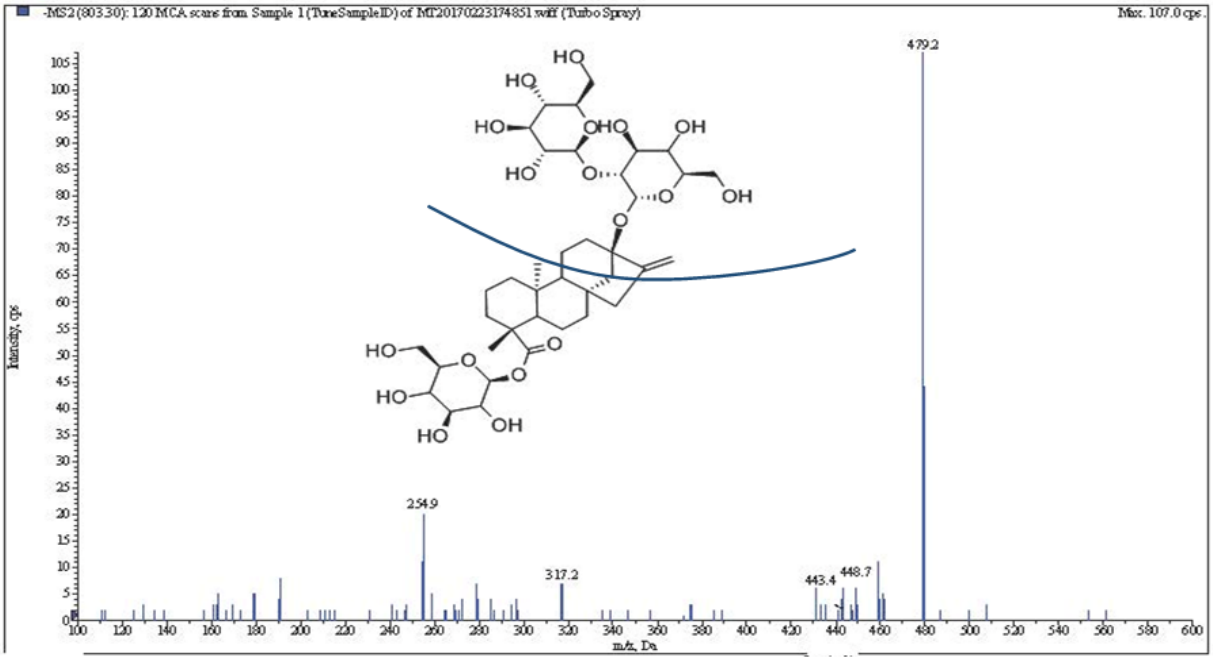

Figure 3 Q3 scan (product ion) spectra B.

Conclusion

This Mass Spectrometer qualitative method proved the presence of Stevia extracted from Armenian leafs. Moreover, this method can be the base for further quantitative Liquid Chromatography tandem Mass Spectrometry methods to quantify the concentration of Stevia in various bushes.

Acknowledgements

None.

Funding

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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