Elemental analysis of fulvic acids of Shilajit using ultra high resolution mass spectrometry

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Khalid Anwer,[1] Matthias Witt,[2] Boris Koch,[3] Suraj Agarwal,[1] Asgar Ali,[1] Jasmin Sultana[1] and Rajesh Khanna[1]

[1] Jamia Hamdard, Hamdrad University, New Delhi, India.
[2] Bruker Daltonik GmbH, Bremen, Germany.
[3] Alfred-Wegener Institute for Polar and Marine Research Bremerhaven, Germany.

Introduction

Shilajit is a pale-brown exudation of variable consistency, oozing out from layer of rocks in many mountain ranges of the world. It originates from bio-organic material having been exposed to high pressures and high temperatures. Shilajit has been used as a rejuvenator and an adaptogen for thousands of years. Its major physiological action has been attributed to the presence of bioactive dibenzo-alpha-pyrons along with humic and fulvic acids which act as carrier molecules for the active ingredients. Fulvic acids are known as a tremendously complex mixture of organic compounds. However, due to the extremely high resolving power and mass accuracy of Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry the elemental composition of these compounds can be determined directly from this complex mixture.

Methods

- Mass spectrometer: APEX Qe 9.4 T (Bruker) using negative ionization.
- Sample: Fulvic acids of Shilajit after extraction with macrophorous ion-exchange resin and desalting with styrene divinyl benzene polymer adsorber.
- Spray Solution: approx. 0.5 mg/mL in water/methanol 50/50 sprayed.

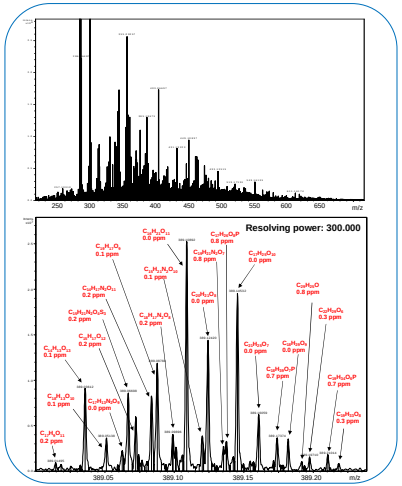

Fig. 1: Full and zoom mass spectrum of Fulvic acids from Shilajit using electrospray negative ion mode

Results

Figure 1 displays the complexity of the sample. Automated assignment of molecular formulas was carried out using the software TargetAnalysis.
Figure 2 illustrates the need for high resolution mass spectrometry: A resolution of 100.000 is insufficient to completely resolve all present compounds.

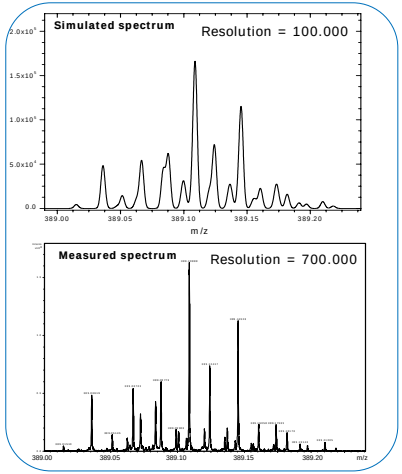

Fig. 2: Comparison of simulated and measured high resolution spectrum at m/z 389

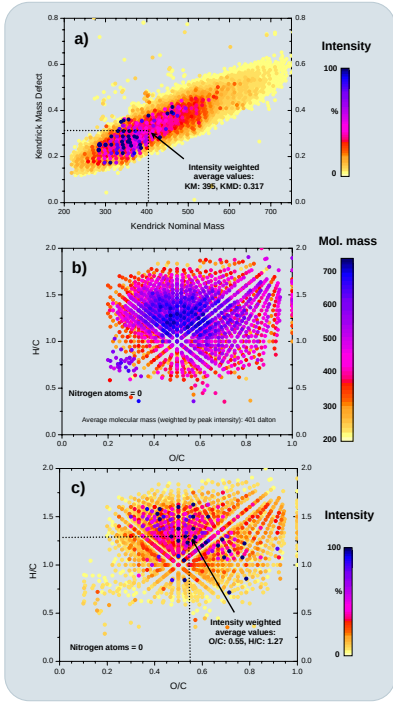

Fig. 3: a) Kendrick Intensity plot, b) Van Krevelen Molecular mass plot and c) Van Krevelen Intensity plot

Table 1: Summary of characteristics of fulvic acids from Shilajit

Analysis Type	Result
Average molecular mass (N=0)	401 Da
Average O/C (N=0)	0.55
Average H/C (N=0)	1.27
Average DBE (N=0)	7.7
Identified elemental compositions (N=0)	2540
Identified elemental compositions (N=1)	1566
Identified elemental compositions (N=2)	1071
Elemental CHNO analysis by FTMS using ESI negative ion mode	C (%): 54.40 H (%): 5.67 N (%): 1.14 O (%): 38.79

Conclusions

- More than 5000 elemental compositions could be identified in the fulvic acid extract of shilajit.
- Average H/C ratio of 1.27 and O/C ratio of 0.55 are extraordinary high compared to Suwannee river fulvic acids standard from the IHSS.
- The Van Krevelen plot shows numerous molecular formulas with O/C > 0.8 possibly indicating the presence of carbohydrates.

ESI FT-ICR MS