

## Molecular composition - inhibition activity relationships for humic substances narrow fractions sets obtained by solid-phase extraction

Mikhnevich T. A.,<sup>1</sup> Bezpalaya E. Y.,<sup>1</sup> Grigorenko V. G.,<sup>1</sup> Burykina Y. V.,<sup>2</sup> and Perminova I. V.<sup>1</sup>

<sup>1</sup>Lomonosov Moscow State University, Department of Chemistry, Moscow, Russia,  
tanya.4erkasova8q@gmail.com

<sup>2</sup>Zelinsky Institute of Organic Chemistry of RAS, Moscow, Russia

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Humic substances (HS) have a wide spectrum of biological activity including inhibitory activity against  $\beta$ -lactamases.<sup>1</sup> The latter are capable of hydrolyzing beta-lactam antibiotics and represent one of the main pathways of bacterial antibiotic resistance. HS are characterized by low toxicity and good solubility in water. A use of HS for therapeutic purposes is hindered by extreme molecular heterogeneity and high level of isomeric complexity. Solid-phase extraction (SPE) fractionation in combination with ultra-high resolution mass spectrometry (FTICR MS) is a promising method to simplify this molecular system and isolate the most active components of HS. The aim of this work was to test various SPE fractionation schemes as an approach to directed isolation of the components with the given activity from HS.

The sample of coal humic acids (CHA-G) was isolated from the commercial sodium humate "Genesis" and separated using SPE cartridge according to gradients in polarity<sup>1</sup> and acidity<sup>2</sup> inherent within the molecular components of HS. Inhibitory activity against  $\beta$ -lactamase TEM-1 and its mutants was measured using chromogenic substrate CENTA. Molecular composition of fractions was determined using FTICR mass spectrometer 15 T solariX (Bruker Daltonics) located at the Collective Use Center of Zelinsky Institute of Organic Chemistry of RAS. Molecular assignments were plotted into van Krevelen diagrams. The diagrams were binned into 20 cells are assigned to seven chemotypes, and occupational densities for each chemotype were calculated after Perminova.<sup>3</sup>

For the fractions separated by polarity, a substantial difference in the molecular composition was observed. Inhibitory activity grew along with an increase in hydrophobicity. The HS activity increased along with an increase in contribution of condensed tannins and phenylisopropanoids ( $O/C < 0.5$ ,  $H/C < 1.4$ ) and decreased along with contribution of hydrolyzed tannins ( $O/C > 0.5$ ,  $H/C < 1.4$ ). The similar analysis was conducted for the fractions separated with regard to pKa value of the dominating functional groups. The most isomeric complex molecular components were defined, which can be found in different HS fractions, but they are identical in elemental composition. The data obtained make it possible to choose the most efficient fractionation method that effectively lowers the molecular complexity of HS and makes it possible to isolate the most active HS fractions.

SPE-fractionation in combination with 2D chromatography is going to be used in our future studies to achieve high resolution separation and more reliable "molecular composition-activity" relationships. Further research might bring substantial advance in the field of directed design of biologically active humic-based materials and compositions.

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## References

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