

Comprehensive HRMS chemical characterization of an antioxidant drink via a newly developed suspect and target screening workflow

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Antioxidants play a significant role in human health, protecting against a variety of diseases including cancer, ageing, neurodegenerative diseases, etc. The augmentation of antioxidant capacity in humans is highly desirable, so the development of products with antioxidant activity is becoming increasingly prominent in human lifestyle habits. In this direction, a new antioxidant drink containing pomegranate, red berries, red grapes, and aronia was designed by the ASOP corporation.

The comprehensive characterization of the drink's constituents has been deemed necessary in order to evaluate its bioactivity. The matrix of our interest consisted of a plethora of substances in a wide range of concentrations. Thus, a High Resolution Mass Spectrometry technique has been selected due to the combination of its sensitivity and structure identification capability. A reversed-phase (RP) chromatographic method hyphenated with a time-of-flight mass spectrometer (LC-qTOFMS and Tims-TOF -Pro2, Bruker Daltonics) has been employed in both ionization modes (\pm ESI). Data dependent (DDA/AutoMS) and data independent (DIA/bbCID) acquisition modes have been utilized in conjunction with open-source software to ascertain the mining of the maximum number of plausible candidates with the lowest false discovery rate.

The obtained data have been treated according to a novel, newly designed workflow based on MS-DIAL for suspect, as well as target screening. This software is utilized for annotation of metabolites in an untargeted mode. However, the classical workflow proposed by MS-DIAL team has been modified in order to be compatible with suspect and target screening in automatic way. The methodology was based on the import of an in-house library, compiled of spectra either from reference standards existing in the laboratory or entries from curated public mass spectral libraries such as MoNA, GNPS, Coconut, MassBank etc.

Compounds belonging to ontologies with possible antioxidant capacity have been identified, such as flavonoids, amino acids, and fatty acids, that could be beneficial to humans' health, revealing the importance of the produce drink as well as the efficacy of the new in-house developed workflow.

Keywords: superfruits, antioxidants, HRMS, target and suspect screening, MS-DIAL

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