

# Rapid MALDI-TOF-MS detection of anthocyanins in wine

## Macedonia

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) as a new valuable technique can have a great impact on wine studies because of the opportunity for rapid qualitative screening of polyphenolic compounds. This study by Violeta Ivanova (Institute of Chemistry, Faculty of Natural Sciences and Mathematics and Department for Enology, Institute of Agriculture, Ss Cyril and Methodius University, - Skopje, Macedonia) and colleagues was performed in order to evaluate and find the most appropriate matrix for analysis of anthocyanins in wine samples and grape extracts and then to develop an efficient MALDI-TOF-MS method for screening of anthocyanins in these samples (*Food Analytical Methods*, DOI 10.1007/s12161-010-9143-7). For that purpose, the efficiency of different MALDI matrices, such as  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA), 2,5-dihydroxybenzoic acid (2,5-DHB) and C70 fullerene, was checked (Fig. 1).

This research work reports a simple and fast method for anthocyanin identification in grape extracts and wine, considered as the key compounds in enology and viticulture necessary to be monitored.

"2,5-DHB was superior with respect to all the matrices used in the study and allowed identification of glucoside, acetylglucoside and p-coumaroylglucoside derivatives of delphinidin, cyanidin, petunidin, peonidin and malvidin," stated Dr Ivanova.

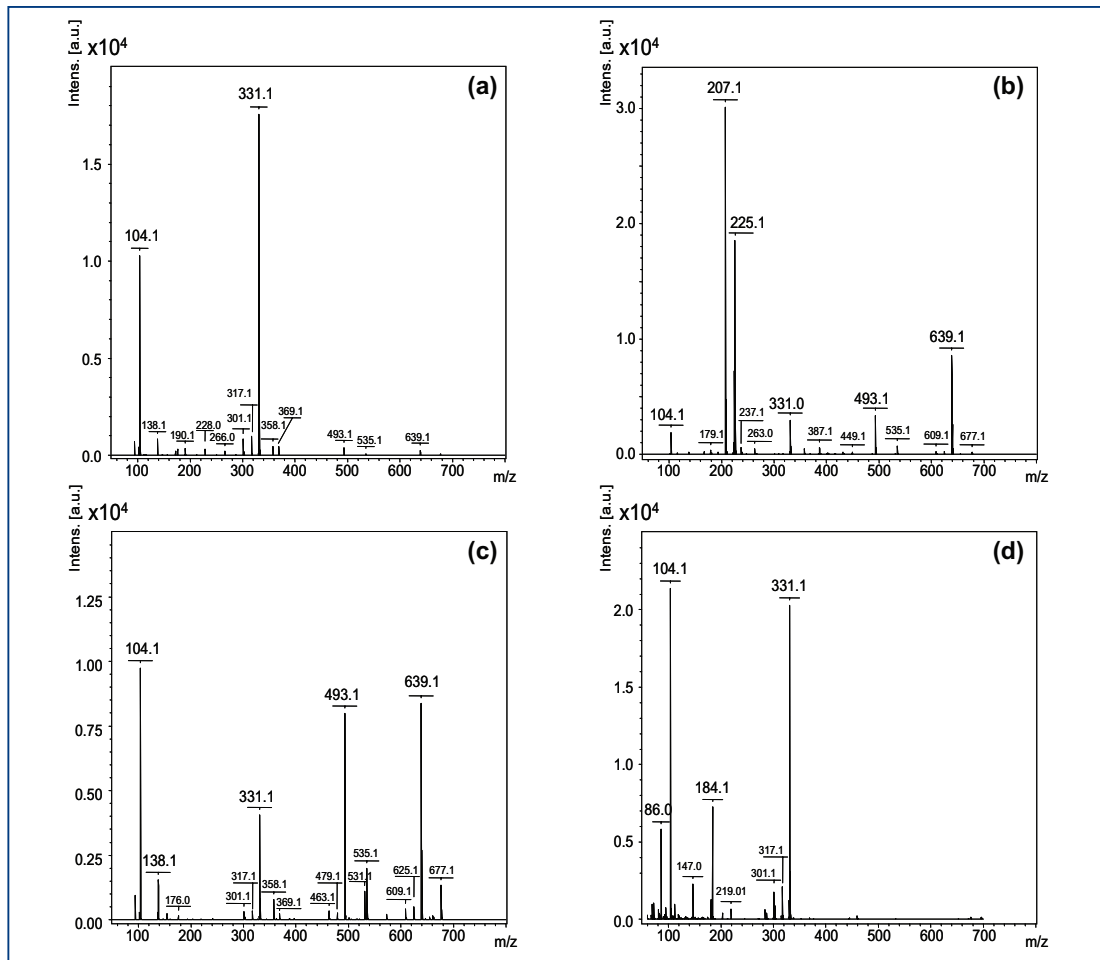
"Fullerene was for the first time tested and used as a possible matrix for anthocyanin analyses, applying the 'sandwich' crystallization method," Ivanova explained. However, anthocyanins were identified only in their aglycone forms, as higher laser energy was necessary for the ionization of the matrix; i.e., the fullerene molecules and led to a great deal of fragmentation of the sugar moiety from glucosides, acetylglucoside and p-coumaroylglucoside groups from the corresponding anthocyanin molecules." Thus, it was concluded

that further extended investigations should be performed on testing derivatized fullerenes, as a potential new matrices suitable for MALDI-TOF-MS analysis of anthocyanins," she continued. "Skin extracts and wine samples were analysed without additional purification confirming the ability of MALDI-TOF-MS to analyse crude samples. In addition, LC-MS analysis has been performed in order to confirm the obtained MALDI-MS results for the presence of anthocyanins in the skin extract and wines."

MALDI-TOF-MS technique allows successful identification of the anthocyanin molecules in complex samples, such as wine, without previous isolation or clean-up of the sample. Ivanova concluded, "MALDI-TOF reduces the analysis time, taking only a few minutes, compared with traditional LC-MS methods, and thus demonstrates its ability as valuable technique for fast screening."

The same team of authors has already performed research, applying MALDI-TOF method after solid-phase extraction of pigments from





Positive-ion MALDI-TOF mass spectra of the grape skin extract from Vranec variety obtained with different MALDI matrices: (a) (α-cyano-4-hydroxycinnamic acid), (b) SA (sinapic acid), (c) 2,5-DHB (2,5-dihydroxybenzoic acid) and (d) C70 fullerene („sandwich” method)

wine for studying a broader range of anthocyanins and their derivatives. In this study, 2',4',6'-trihydroxyacetophenone (dissolved in acetonitrile/H<sub>2</sub>O = 1/1) was used as a matrix for wine MALDI-TOF-MS screening. Before MALDI analysis, solid-phase extraction of the pigments was performed using new Zip-Tip pipette tips filled with C18 stationary phase. MALDI-TOF-MS results showed the presence of glucoside, acetylglucoside and p-coumaroylglucoside derivatives of delphinidin, cyanidin, petunidin, peonidin and malvidin in the wine extracts. Furthermore, pyranoanthocyanins formed by reactions of anthocyanins with pyruvic acid and acetaldehyde, as well as flavan-3-ol-anthocyanin adducts and ethyl-bridged pigments have been isolated and identified in the extracts.

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