

COMPARISON OF TWO AZOBENZENE-BASED AMINO ACID DERIVATIZATION REAGENTS FOR LC-MS/MS ANALYSIS IN POSITIVE AND NEGATIVE ESI MODES

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Derivatization reagents based on azobenzene: AzoB (Azobenzene N-hydroxysuccinimidyl carbamate) and AzoC (4-(phenylazo)benzoic acid N-succinimidyl ester) are proposed for the analysis of free amino acids in fermented beverages and juices using LC-ESI-MS. A comparison between LC-MS/MS in positive and negative ESI modes in dynamic Multiple Reaction Monitoring (dMRM) and Neutral Loss Scan was investigated.

Derivatization was performed on three different samples: Kali, watermelon juice and tomato juice, using AzoB and AzoC. Analysis of derivatized samples was performed with an Agilent UHPLC-MS system using a biphenyl column.

Both derivatization reagents reacted with primary and secondary amino acids. AzoB showed characteristic fragmentation patterns in positive and negative mode while AzoC only in positive mode. Accuracy of the methods was determined by using a Certified Reference Material (CRM) containing 15 of the 21 analyzed amino acids, it ranged from 91 to 104% (RSD<7%). Both methods were linear in the range from 6.7 to 1300 nmol L⁻¹, $r^2 > 0.993$ for AzoB derivatives and $r^2 > 0.984$ for AzoC derivatives. LoQ values obtained with AzoB (pmol on column), are comparable to those found with AQC, and were all lower than the ones reported when PITC or aTRAQ are used [1].

References

[1] E. de J. Zapata Flores, N.K.N. Bùi, S. Selberg, K. Herodes, I. Leito, Comparison of two azobenzene-based amino acid derivatization reagents for LC-MS/MS analysis in positive and negative ESI modes, *Talanta*. 252 (2023) 123803. <https://doi.org/10.1016/j.talanta.2022.123803>.



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