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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1885387> since 2023-01-12T14:18:07Z

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Detection of amino compounds by diethyl ethoxymethylenemalonate derivatization and neutral loss scan mode

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Keywords: amines, derivatization, diethyl ethoxymethylenemalonate, LC-MS, neutral loss scan mode

Introduction: Amino compounds form a significant part of metabolites and can be found in different matrices (*e.g.* plants, food). Identification of all metabolites is difficult, given the amount and different chemical properties of each [1]. There is no available technique with enough selectivity and sensitivity to englobe the wide range of metabolite diversity. A way to narrow down and target a specific functional group is through derivatization, where a moiety is introduced in the compounds of interest by a chemical reaction. Analysis is carried out with liquid chromatography-tandem mass spectrometry (LC-MS/MS), due to its sensitivity and the fact that it provides information about the molecular mass, thereby enabling analyte identification. Derivatives also have better LC separation and higher sensitivity than the underivatized analytes.

Diethyl ethoxymethylenemalonate (DEEMM) was the amine derivatization reagent of choice in this work, because of the reaction simplicity and the fact that derivatives lose a neutral fragment 46 (ethanol) from the parent ion. In this case, neutral loss scan mode is employed, allowing the detection of all the compounds with primary and secondary amino groups, with the later reacting at a slower rate. Plant material (*Carduus nutans* subsp. *macrocephalus*) was employed as a model sample, and amino compounds were identified for the first time in this species [2].

Methods: Extraction of the plant material was carried out with 5 mL of 30% methanol in 0.1 M HCl out for 20 minutes in an ultrasonic bath at room temperature. The extract was derivatized with DEEMM and hydroxylamine solution was employed as a quenching reagent. Derivatized sample was analysed with LC-ESI-MS in neutral loss scan mode (loss of 46) in the m/z 50-600 range.

Results: Hydroxylamine was chosen as the quenching reagent due to its short retention time and solubility in the employed solvent (30% methanol in 0.1 M HCl). Out of 34 peaks, 18 amino acids and 3 biogenic amines were identified in the plant material by the injection of reference standard substances. Three compounds were putatively identified according to the literature.

Conclusions: Neutral loss scan mode after DEEMM derivatization and hydroxylamine quenching proved to be a successful procedure to identify amines in a plant extract. This method can be applied to different matrices.

Acknowledgments: Support by the Estonian Research Council grant PUT1589, by the EU through the European Regional Development Fund (TK141 “Advanced materials and high-

technology devices for energy recuperation systems”) and was carried out using the instrumentation at the Estonian Center of Analytical Chemistry.

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