

# UHPLC-MS/MS METHOD DEVELOPMENT FOR TARGETED GIBBERELLIN PROFILING IN REPRODUCTIVE TISSUES OF HERACLEUM SOSNOWSKYI

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Gibberellins (GAs) are a large group of plant hormones found in trace quantities that are involved in the control of plant growth and development [1–4]. *Sosnowsky's* hogweed (*Heracleum sosnowskyi* Manden.) is listed among Invasive Alien Species that are of high concern to the EU [5]. Concern is raised by its ability to destroy native ecosystems and by its toxicity to human health. Spreading occurs only by seeds, and death occurs after reproduction. For seed development and further spread, a crucial role is played by GAs [6]. Therefore, valuable information about the state of GA metabolism in response to various treatments or changes in environmental conditions is provided by the quantification of endogenous GAs in *H. sosnowskyi* tissues [2]. Thus, the efficient extraction, purification, and determination of minute GA amounts are essential [2]. In this study, the development of an UHPLC–MS/MS method for the quantification of GAs in *H. sosnowskyi* reproductive tissues is aimed at, and the optimization of an established GA extraction and purification protocol is performed.

In this study, a selective and sensitive UHPLC–MS/MS method was developed for the separation and quantification of 14 different GAs from 100 mg of *H. sosnowskyi* reproductive tissues. Optimal separation of GAs was achieved on a UHPLC reversed-phase column (ReproSil-Pur Basic-C18, 2.1 mm x 100 mm, 1.9 μm; Dr. Maisch) using a linear gradient of 0.1% formic acid (A) and acetonitrile (B) mobile phases at a flow rate of 0.4 mL min<sup>-1</sup>, from 95:5 A:B (v/v) to 30:70 A:B (v/v) over 13 min, with column washing performed using 0:100 A:B (v/v) until 15 min. Quantification of GAs was performed using a highly selective multiple reaction monitoring (MRM) mode and an isotope dilution method. Furthermore, an existing GA extraction and purification method developed by Urbanova et al. [2] was modified and applied. The method is based on solid-phase extraction (SPE) with mixed-mode (Oasis MCX; Oasis MAX, Waters) and reversed-phase (Oasis HLB, Waters) cartridges. Insufficient recoveries of GAs for quantification were obtained from extraction using the originally developed protocol. Therefore, the method was modified, and improved recovery of GAs was achieved by adjustment of the composition of solvents used in SPE.

In conclusion, a robust UHPLC–MS/MS method was developed for the quantification of GAs in *H. sosnowskyi* reproductive tissues, and an extraction and purification protocol was optimized that provides a foundation for further investigations into GA-mediated regulation of reproductive development in this species.

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