Metabolite Profiling of the Monolignol Biosynthesis Pathway using Liquid Chromatography coupled with Tandem Mass Spectrometry

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Monolignols constitute the fundamental units of lignin, which impart structural strength, vascular solidarity and pathogen resistance to plants. Thus, monolignols largely determine the composition and quality of lignin. It has been observed that altering lignin synthesis improves conversion efficiency of biomass into energy, food or other industrial materials. As a result, a lot of recent research efforts have been invested in understanding the underlying mechanism of monolignol biosynthesis, making the quantification of the intermediates of the pathway invaluable. We have developed a novel comprehensive method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the determination of the intermediates of the monolignol biosynthetic pathway.

The experimental procedure entailed extraction of metabolites from *Arabidopsis thaliana* stem tissue using methanol and water as solvents. The extract was then analyzed using reverse phase LC-MS/MS by electrospray ionization (negative ion mode) with multiple reaction monitoring (MRM) of two ions (Q1–parent/Q3–daughter) per compound. The analytical method was optimized with respect to buffer concentrations, flow rate, pH of mobile phase, temperature and extraction protocols. Isotopic feeding experiments with [ring-$^{13}C_6$]-phenylalanine (pathway precursor) were conducted for validation of the method. Previously, profiling these metabolites using gas chromatography coupled mass spectrometry (GC-MS) was attempted. Due to lack of selective reaction monitoring, the abundances of the parent-daughter ions couldn't be effectively used for quantification. Contribution from other extracted metabolites to the ions under consideration was suspected.

13 of the 18 important compounds of the monolignol biosynthetic pathway were detected and quantified. Limits of detection (LODs) and quantification (LOQs) were obtained for all metabolites. Cinnamic acid, the second intermediate of the pathway, was found to be below the detection limits of the method in *A. thaliana* tissue. Ion-suppression was studied and accounted for by using a set of internal standards (ISs) and conducting spiking studies with corresponding standards. Large matrix effects on the final peak intensities of the metabolites were observed. Apart from mainstream intermediates of the pathway, our method detected prominent alternative secondary metabolites like sinapoyl malate and sinapoyl glucose.