Characterization of diastereoisomeric flavonol glycosides by liquid chromatography multiple stage mass spectrometry assisted by chemical modifications

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Introduction: Flavonol glycosides are important components from leaves of vascular plants. However, a lot of isomers are reported, making their analysis difficult, leading to many structural misinterpretations. Galactosides and glucosides alone or in oligosaccharides are components of many diastereoisomers, being indistinguishable to mass spectrometry. To resolve this, isomeric flavonol glycosides were chemically modified and submitted to liquid chromatography multi-stage mass spectrometry (LC-MSⁿ) analysis. Material and Methods: Standards of β-methyl-glucoside and β-methyl-galactoside were converted to 4,6-isopropylidene β-methyl-glucoside and 3,4-isopropylidene β-methyl-galactoside employing anhydrous acetone and p-toluenesulfonic acid. These derivatives were analyzed by offline tandem mass spectrometry to provide a fingerprint of them. Further, the method was employed to extracts from leaves of Maytenus ilicifolia and Camellia sinensis, known to contain isomers of flavonol glycosides, which were analyzed by liquid chromatography-mass spectrometry. Results and Discussion: The chemical modification was consisted by a simple step of O-isopropylidene (IPP) formation on the suitable hydroxyl groups. When the glycoside allowed the formation of IPP-derivative, the mass of the compound shifted in 40 mass units for each ketal group introduced. When glucose was substituted in position C-6, the ketal was prevented, but this does not occurs with galactosides, giving a product with 40 mass units greater than glucosides. In cases where both isomers allowed IPP-ketal formation, multi-stage tandem was used to isolate the specific fragment of IPP-monosaccharide residue, which was fragmented and compared with the standard fingerprints allowing their identification. Also, the reaction decreased the polarity of the compounds, improving the chromatographic interaction with reversed phase chromatography. Conclusion: The isopropylidene method allowed the identification of many isomers direct by offline mass spectrometry, since the derivatization provided compounds with different molecular weight. Those compounds not distinguished directly by offline mass spectrometry, could be differentiated by LC-MSⁿ because the reaction provided different MS-fingerprints for derivatized glucose and galactose.

Keywords: Flavonol glycoside isomers, isopropylidene ketal, multi-stage mass spectrometry, Camellia sinensis, Maytenus ilicifolia.

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