

## LC and GC hyphenated to Mass Spectrometry as Tool for Characterization of unknown Isoflavonoids

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Isoflavones (IF) belonging to the class of polyphenolic secondary plant metabolites play an important role in human nutrition as bioactive food constituents especially in dietary supplements. Structure elucidation of IF and other polyphenols in plant extracts or metabolites, especially those arising during biotransformation is still a challenge in analytical chemistry. Various approaches have been proposed to utilize mass spectrometric fragmentation reactions for structural elucidation on polyphenolic targets. It is known that these substances undergo an intense fragmentation process in both LC-ESI-MS and GC-(EI)-MS. We combine the information from both techniques for structure elucidation of IF, utilizing already established IF fragmentation reactions as well as fragmentation patterns which have not been described yet.

**Two LC-ESI(+)-MS/MS fragmentations** based on different C-ring cleavages which are characteristic for flavonoids are presented. 1.) The basic retro-Diels-Alder (rDA) fragmentation, which offers information about the substitution pattern in the A- and B-ring of flavonoids. 2.) The elimination of a protonated 4-methylphenol cation ( $m/z$  107), which is used as a diagnostic tool for the structure elucidation of isoflavanones. **Three fragmentation reactions** are presented for **GC-(EI)-MS/MS** analysis after silylation of the analytes. All of them are based on the elimination of methyl radicals or tetramethylsilane groups under formation of stabilized siloxane rings. The specificity of all of these fragmentations has been demonstrated with the help of authentic reference compounds [1].

Here, the fragmentation reactions are exemplarily applied on two unknown metabolites of the IF irilone (IRI). We demonstrate how the combination of fragmentation reactions in both techniques can lead to a reliable structure elucidation. Thus, we are able to present the structure of a reductive as well as a hydroxylated derivative of IRI. IRI as well as the metabolites are bearing a methylenedioxygroup and therefore are toxicologically interesting components of red clover based dietary supplements.

Additionally, our results are transferable on various polyphenols, for example to distinguish between a methylated and dihydrogenated (+ 16 amu) and a hydroxylated (+ 16 amu) metabolite of the same parent flavonoid. Thus, the presented reactions represent a useful analytical approach to obtain structural information about substances being relevant in the metabolism or new secondary plant metabolites. Results from a single mass spectrometric technique always leave doubts, thus the confirmation with a second independent method is necessary for structure elucidation. This provides enough certainty in structural analysis and therefore does not require increased concentration levels sufficient for NMR analysis.

[1] R. Maul, N.H.Schebb and S.E. Kulling, Anal. Bioanal.Chem (2008), in press (DOI 10.1007/s00216-008-1884-4)