Structure elucidation of isoflavanoides by fragmentation reactions in mass spectrometry hyphenated to LC and GC

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Polyphenolic secondary plant metabolites play an important role in human nutrition. In particular isoflavones (IF) are important bioactive food constituents in dietary supplements. It is still a challenge in food chemistry to identify the IF in foodstuff or those derivatives arising during biotransformation. Therefore, methods for structure elucidation of IF and other polyphenols are needed. Various approaches have been proposed to utilize mass spectrometric fragmentation reactions for structural considerations on polyphenolic targets. It is known that these substances undergo an intense fragmentation process in both GC-(EI)-MS and LC-ESI-MS. Recently, we developed a method for structure elucidation of IF by combining the information from both techniques [1]. It incorporates already established IF fragmentation reactions as well as fragmentation patterns which had not been described before.

Two **LC-ESI(+)-MS/MS** fragmentations based on different C-ring cleavages which are characteristic for flavonoids are presented. I) The basic retro-Diels-Alder (rDA) fragmentation, which offers information about the substitution pattern in the A- and B-ring of flavonoids. II) 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 methylated derivative of IRI. IRI as well as the metabolites are bearing a methylenedioxygroup and therefore are toxicologically relevant food constituents of red clover based dietary supplements.
The present fragmentation reactions are transferable on various polyphenols, for example to distinguish between different methylated, dihydrogenated (+ 16 amu) and hydroxylated (+ 16 amu) metabolites of a known parent compound. Thus, the presented reactions outline a useful approach to obtain structural information about substances being relevant in food analysis. For example new secondary plant constituents or metabolites formed during biotransformation can be screened.
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)