

High Throughput Characterisation of Natural Products Using Multipump Control LC-MS Exact Mass Measurement.

M McCullagh¹, C A M Pereira² and J H Yariwake²

¹Micromass UK Ltd, Floats Rd, Wythenshawe, Manchester, M23 9LZ, UK.

²Universidade de Sao Paulo – Instituto de Quimica de Sao Carlos – P.O Box 780, 13560-970, Sao Carlos – SP – Brazil.

Passiflora species are used as phytomedicines in Brazil due to the sedative properties that are related to the presence of flavonoids in leaves. Due to the importance of flavonoids and their glycosides to these species, the identification and/or structural determination of such compounds occurring in leaves play an important role. As the analysis of natural products from crude plant extracts using LC-MS and LC-MS-MS is becoming more routine, a study of the parameters required using a orthogonal axis time of flight mass spectrometer to provide an efficient route to the dereplication of C-glycosidic flavonoids extracted from Brazilian *Passiflora* species has been performed. Such natural product extracts can contain complex mixtures of numerous flavonoid isomers. Due to this sample complexity, the application of in source CID in combination with exact mass measurement provides a route to specific unambiguous identification. The comparison of pseudo MS-MS fragment ion spectra of flavonoid C-glycoside isomers allows for efficient differentiation. Characteristic differences between the 6-C and 8-C flavonoid glycosides have enabled the identification of such flavonoid isomers. This further allows for the specific identification of the species from which the flavonoids have been extracted.

LC-MS sample analysis has been performed using an orthogonal acceleration time of flight (oa-TOF) mass spectrometer equipped with electrospray ionisation. Analysis has been performed in both positive and negative electrospray ionisation mode. The application of increasing cone voltages to flavonoid standards provided characteristic fragment ion information, where the relative abundance of fragment ions was different for different flavonoid isomers. The increased specificity of exact mass measurement allowed efficient assignment of the fragments using the elemental composition calculator. The experimental conditions determined were applied to different Brazilian *Passiflora* species. Each species was successfully characterised and identified.

As a result of the nature of natural product extracts containing non-polar and polar compounds long HPLC gradients analysis times result, in order to achieve better analyte separation. In the data presented the analysis time was one hour. For the analysis of four flavonoid standards and four *Passiflora* extracts a total analysis time of four hours was required for LC-MS data to be acquired. In order to increase productivity, a 5-Way MUX source was interfaced to the oa-TOF allowing the independently pumped eluents from 4 LC columns to be analysed in parallel on the same mass spectrometer. This reduced the analysis time to one hour for four samples, 25% previous analysis time.