Analysis of isomeric pyrrolizidine alkaloids by online multiple heart-cutting 2D-LC QToF-MS

Milou G.M. van de Schans[1], Marco H. Blokland[1], Patrick P.J. Mulder[1], Paul W. Zoontjes[1], Saskia S. Sterk[1], Michel W.F. Nielen[1,2]

Introduction

Pyrrolizidine alkaloids (PAs) are an important class of secondary plant metabolites that exhibit a wide structural variety including many isomers (Figure 1). Consumption of food products containing PAs (*e.g.* tea, honey) can result in toxic effects. It is known that different PAs can have different toxicities[1], but it is difficult to determine the total toxicity of an extract if not all isomeric PAs can be separated. Currently, PAs are analysed using LC with either high pH[2] or low pH[3], but none of the existing methods can separate all PAs. 2D-LC is a chromatographic technique which could allow for the separation of all isomeric PAs in a single run, since different stationary and mobile phases can be used in the different dimensions.

Objective

To improve the separation of isomeric pyrrolizidine alkaloids which have similar MS fragmentation patterns using 2D-LC QToF-MS.

Set-up of the multiple heart-cutting 2D-LC QToF-MS

In this research a commercially available 2D-LC system is used. The 2D-LC QToF-MS system consists of a pump and column in both dimensions, a DAD-detector in the first dimension and a QToF-MS in the second dimension (Figure 2). The effluent of the first dimension can be transferred to different loops (time based) positioned on two different decks and subsequently injected on the second column. This technique is called multiple-heart cutting.

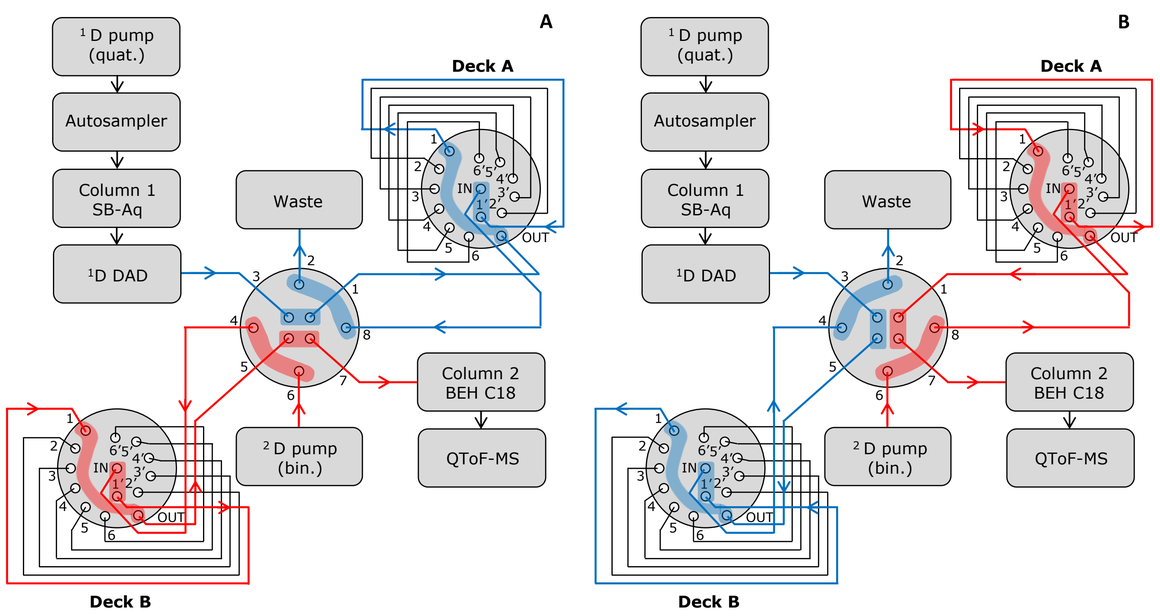

Figure 2. Set-ups of the 2D-LC QToF-MS in multiple heart-cutting mode in which deck A is filled and deck B is analysed (A) or deck B is filled and deck A is analysed (B).

Seven different columns with different retention mechanisms (*e.g.* hydrophobicity) and two different mobile phases were tested (pH 3 and pH 8) to characterise the elution behaviour of the four sets of isomeric PAs (Figure 1). The highest orthogonality was achieved by the use of a Zorbax UPLC SB-Aq column at pH 3 and an Acquity UPLC BEH C_{18} column at pH 8.

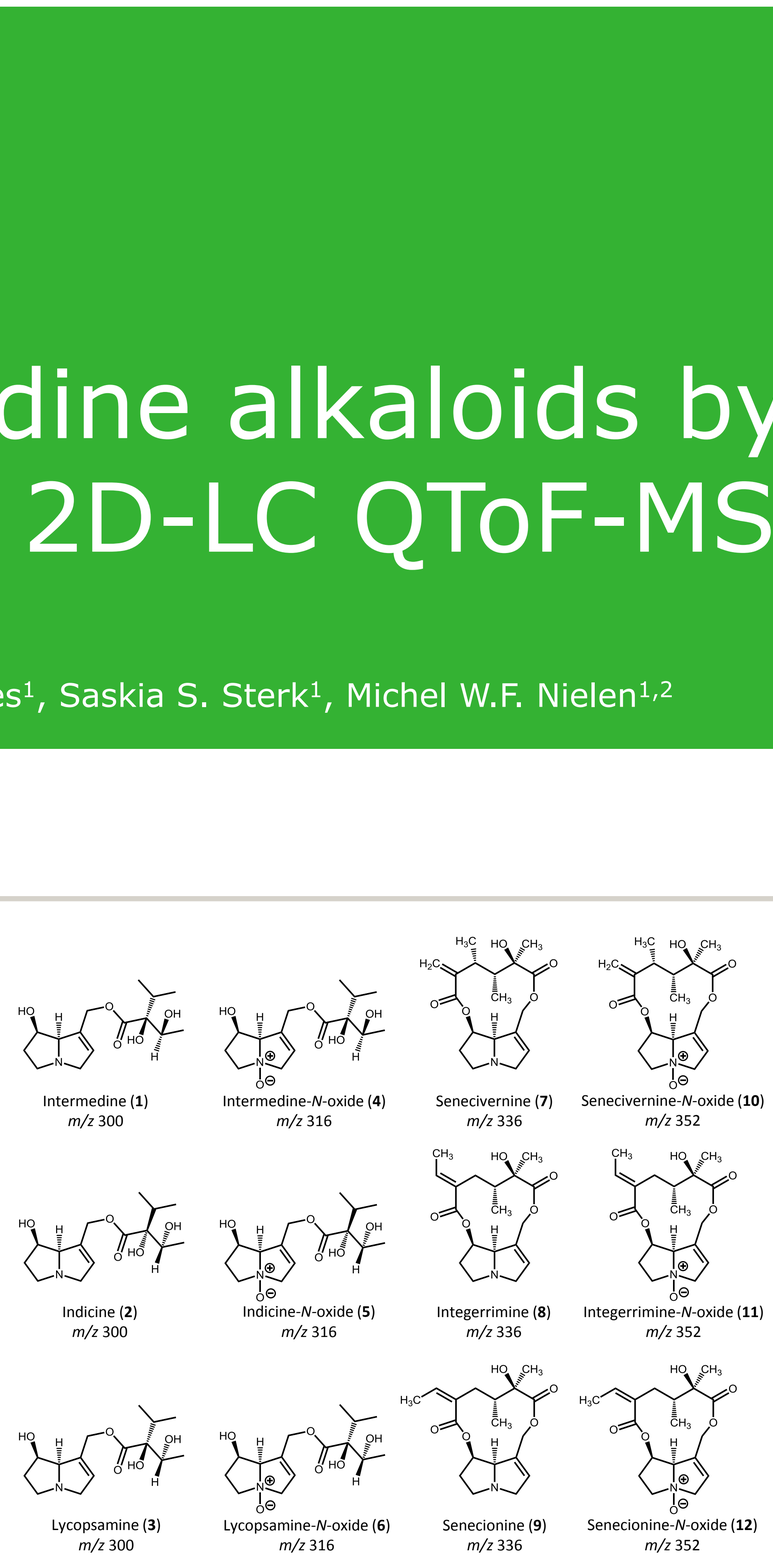

Figure 1. Structures of different pyrrolizidine alkaloids.

Separation of pyrrolizidine alkaloids

Sets of PAs (4-5, 7-9 and 10-12) which could not be separated in the first dimension were stored in the loops and transferred to the second dimension (Figure 3A). For the different sets, different segment (collection) times were used. In Figure 3B+C it can be seen that the co-eluting compounds 4+5 and 7-9 can be separated in the second dimension. Compounds 10-12 could be partly separated in the first dimension and partly separated in the second dimension (Figure 3A+D).

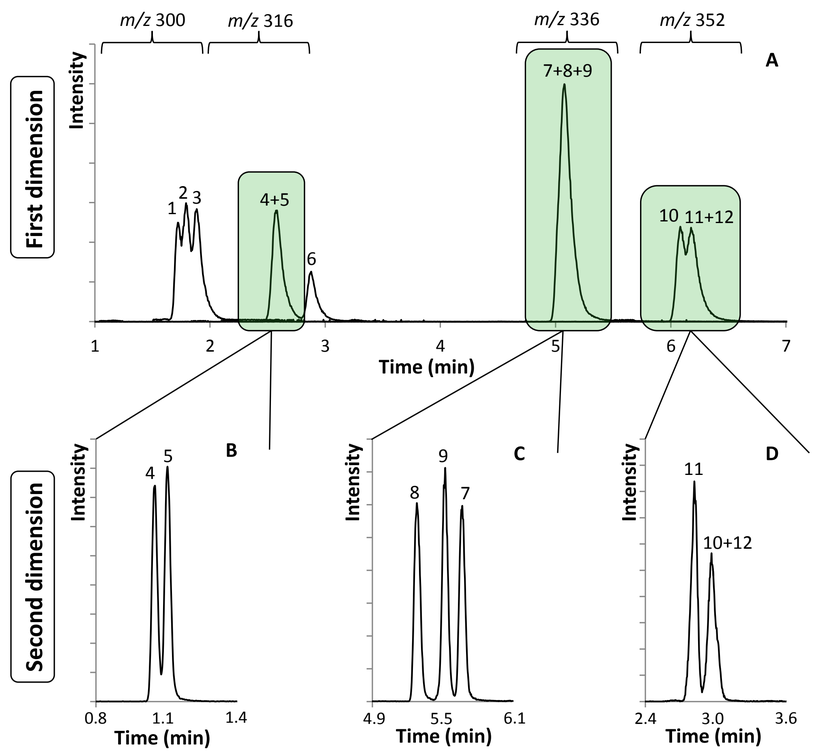

Figure 3. EIC MS-chromatogram of the PAs in the low-pH first dimension (A) and in the time segments in the high-pH second dimension. Segment times: 2.5-2.8 min (B), 5.0-5.3 min (C), 6.0-6.3 (D).

Conclusions

All 12 PAs can be separated in a single run by combining two chromatographic dimensions using online multiple heart-cutting 2D-LC QToF-MS. Next, this application will be tested for the analysis of isomeric PAs present in extracts of food products.

[1] RIKILT Wageningen UR
P.O. Box 230, 6700 AA Wageningen,
The Netherlands
Contact: milou.vandeschans@wur.nl
www.wageningenUR.nl/en/rikilt

[2] Wageningen University
Laboratory of Organic Chemistry
Dreijenplein 8, 6703 HB Wageningen,
The Netherlands

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