An automated method to study the rapid intramolecular transacylation of drug acyl glucuronides using Cyclic Ion Mobility Spectrometry-Mass Spectrometry

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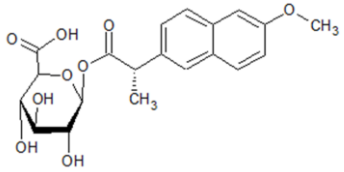
Introduction

Glucuronidation is a common biotransformation mechanism that occurs for many drugs and other xenobiotics. The formation of acyl glucuronides (AGs) can be a cause for concern as these metabolites have been linked with hepatoxicity and withdrawal from the market. AGs form as the 1-β-O acyl isomers and the rate of transacylation of this to the 2, 3 and 4-O-acyl forms has been used to assess the potential risk. This is traditionally performed using *in-vitro* incubations of the 1-β-O form in buffer followed by LC-MS analysis of the resultant samples. This procedure is time consuming as it requires prior LC method development, and discrete samples for analysis.

The SELECT SERIES™ Cyclic™ IMS has been shown as an excellent alternative to provide real-time monitoring of the transacylation of diclofenac 1-β-O acyl glucuronide[1]. This has been further developed to use the LC autosampler for incubation at 37ºC followed by flow injection analyses (FIA). This automated method has been used to monitor the transacylation of 1-β-O-acyl Naproxen (NAG), demonstrating the capability of the Cyclic IMS (cIM) to provide rapid data with automated incubation and sampling.



1-β-O-acyl Naproxen

Experimental

NAG (100 µM) was incubated in 10mM ammonium acetate (aq.) for 2 hrs at room temperature to provide a mix of isomers for LC and IMS method development.

Real time incubation of NAG was monitored using a fresh incubation with negative ion electrospray Q-IMS-Tof analyses with 5 passes of the cIM.

Results

	M+H⁺	M+HCOO⁻	M+K⁺	M+Na⁺	M-H⁻
1-O-Nag	196.6	197.1	197.1	197.1	197.1
2-O-Nag	196.3	196.8	196.8	196.8	196.8
3-O-Nag	196.3	196.6	196.6	196.6	196.6
4-O-Nag	196.3	196.6	196.6	196.6	196.6

Table 1. Predicted CCS for NAG isomers using CCS OnDemand[2].

This prediction indicated that CCS may allow ion mobility separation of 1-O NAG from the other forms

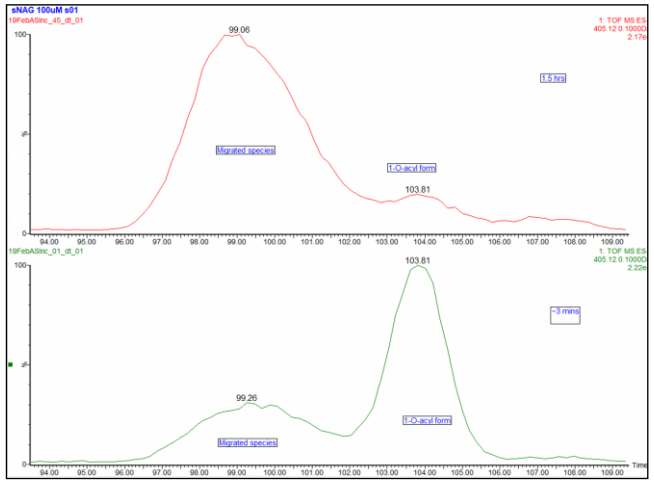


Figure 1. Arrival time distribution (ATD) after 5 passes of the cIM for m/z 405 $[M-H]^-$ following analysis of the 1-β-O NAG incubation sample after 3 min (lower trace) and 1.5 hrs (upper trace) and intact (lower trace).

References

1. Higton. D. et al. Anal Chem, 2021, 93, 20, 7413-7421
2. Broeckling, C et al. J Am Soc Mass Spectrom, 2021, 32, 661-669

Conclusions

The established LC-MS methodology for monitoring acyl migration utilizes ~15 min LC-MS methods, which makes it challenging to study rapidly trans-acylating species. However, this can be overcome by real time monitoring. We have described a novel method that enables calculation of the half-life of the reaction and real time monitoring of acyl migration from incubations undertaken in the LC Sample Manager using flow injection analysis and the high ion mobility separation power of the Waters SELECT SERIES Cyclic IMS. This new approach provides an easier, and significantly faster method for studying migration of acyl glucuronides.

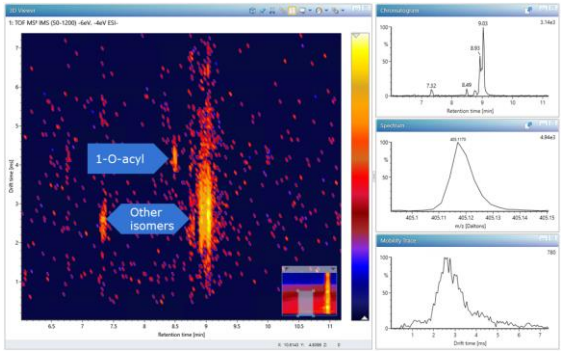


Figure 2. LC-MS analysis with 5 passes of the cIM of 1-β-O NAG after incubation showing disappearance of 1-β-O and appearance of other isomers

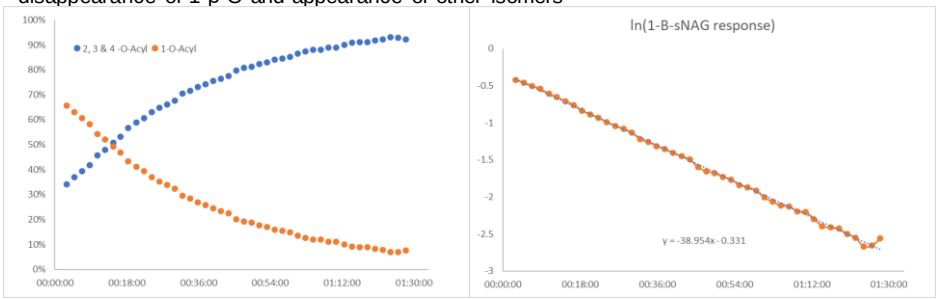


Figure 3. FIA of 1-β-O NAG following incubation showing disappearance of 1-O- form in amber and appearance of other isomers in blue (left) and kinetic plot of 1-β-O NAG with calculation of the half-life (right)