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

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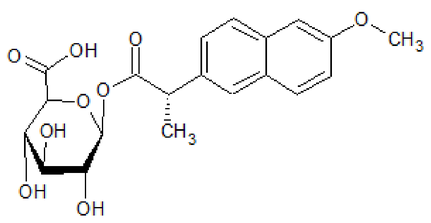
Introduction

Glucuronidation is a common biotransformation mechanism that occurs for many drugs and other xenobiotics, either directly or for phase 1 metabolites. Generally, glucuronide metabolites are not seen to present any safety risk during development to market. The formation of acyl (i.e ester) glucuronides (AGs) with carboxylic acid-containing drugs can be a cause for concern with regulatory authorities[1] as these metabolites have been linked with hepatoxicity. This hepatoxicity has led to the withdrawal of drugs from the market e.g. ref 2. AGs are initially formed as the 1-β-O acyl isomers and the rate of transacylation of this isomer to the 2, 3 and 4-O-acyl forms has been used to assess the potential risk. The conventional approach to study the rate of transacylation is to perform *in-vitro* incubations of the 1-β-O form in buffer followed by LC-MS analysis of the resultant samples e.g. ref 3. This procedure is time consuming as it requires prior development of a LC method, and the discrete samples are needed for analysis.

The SELECT SERIES™ Cyclic IMS, a quadrupole Cyclic Ion Mobility Spectrometry-Mass Spectrometer (cIM-MS) has recently been shown as an alternative method to provide real-time monitoring of the transacylation of diclofenac 1-β-O acyl glucuronide[4]. In this method, ion mobility is used to separate the 1-β-O form from the other species and samples were incubated at room temperature with infusion of samples to monitor the reaction. Here, we have developed this methodology for the incubation experiment by incubating the samples in an LC autosampler at 37°C followed by flow injection analyses. This automated method has been used to monitor the transacylation of 1-β-O-acyl Naproxen, demonstrating the capability of the Cyclic IMS to provide rapid data with automated incubation and sampling.



Structure of 1-β-O-acyl Naproxen

Experimental

LC and Cyclic IMS method development: s-Naproxen1-β-O-acyl glucuronide (sNAG) was prepared at 100µM in MeCN/H_2O + 0.1% HCOOH and 10 mM NH4Ac (pH 7.4) and left at room temp for 2h.

Infusion experiment: 100µM sNAG was prepared on 10 mM NH4Ac (pH 7.4) and placed in the autosampler held at 37°C. Samples were analysed every 2 minutes using flow injection (FIA) at 40 µL/min.

Instrumentation:

Waters™ ACQUITY UPLC ™ I-Class PLUS Systemand SELECT SERIES™ Cyclic IMS

LC Conditions:

Waters™ ACQUITY UPLC® HSS T3, 100x2.1mm,1.8 µm at 40°C
A: 10mM Ammonium Acetate, pH 7.4
B: Acetonitrile

Time(min)	Flow Rate	%A	%B
Initial	0.5	98	2
1.0	0.5	98	2
15.0	0.5	5	95
17.0	0.5	5	95
17.1	0.5	98	2
20.0	0.5	98	2

MS Conditions:

Electrospray: -1.5 kV; Source: 120 °C; Desolvation: 500 °C
Desolvation gas: 800 L/hr
Scan: m/z 50 – 1200 in 0.2 seconds
Ion mobility separation time: 2ms for CCS or 81 ms for infusion with multiple passes

CCS prediction

Prior to performing any experiments, a machine learning model[5] was used to predict the CCS values of the 1-β-O and 2, 3, 4-O isomers of Naproxen to estimate the likelihood that these isomers could be separated using ion mobility.

	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 sNAG isomers

This prediction indicated that CCS for the 1-O form may allow ion mobility separation from the other forms in both positive and negative ion mode.

Results

LC-MS (Figure 1) was used to confirm that the incubation procedure was rapid at room temperature and to allow confirmation that all the isomers were present for method development.

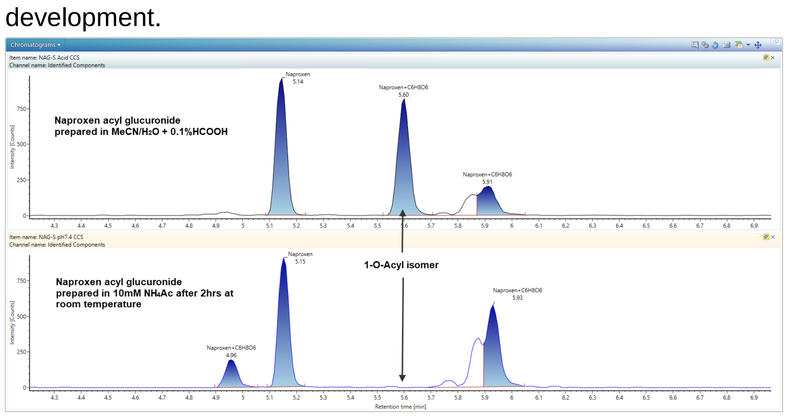


Figure 1. LC-MS analyses of intact (upper trace) and migrated (lower trace)1-β-O sNAG

These samples were then infused into the instrument to develop a cyclic sequence that could separate the 1- β-O isomer from the other forms.

1-β-O sNAG was incubated at 37 ºC in the LC sample manager with an initial sample at 3 minutes, and further samples approximately every 2 minutes until 1.5 hrs. Data (Figure 2) obtained after incubation 1-β-O sNAG for 3 min and 1.5h. These data compare well to the CCS prediction for the 1-O species and is distinguished from the other forms, the other isomers are not separated from each other.

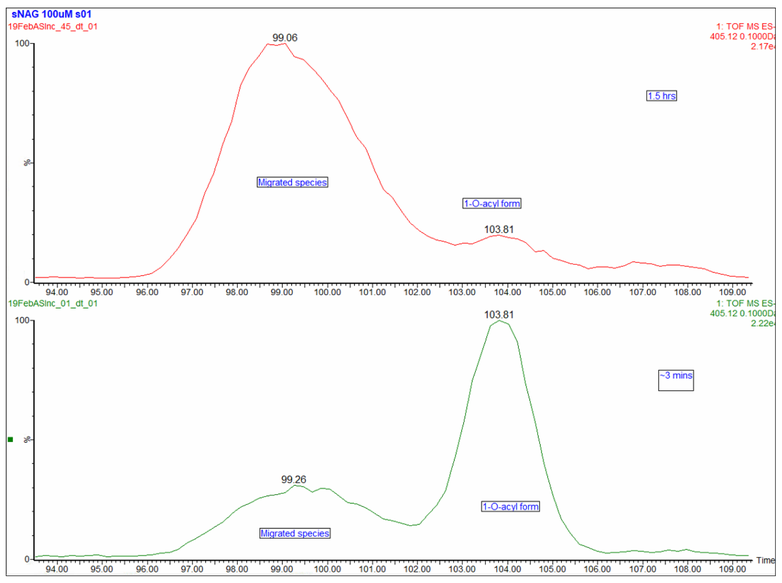


Figure 2. Arrival time distribution (ATD) after 5 passes of the cIM for m/z 405 $[M-H]^-$ following analysis of the 1-β-O sNAG incubation sample after 3 min (lower trace) and 1.5 hrs (upper trace) and intact (lower trace).

The areas of the 1-β-O sNAG and other isomers observed in the ATD were integrated and the disappearance of the 1-β-O sNAG and appearance of the other isomers can be seen in Figure 3.

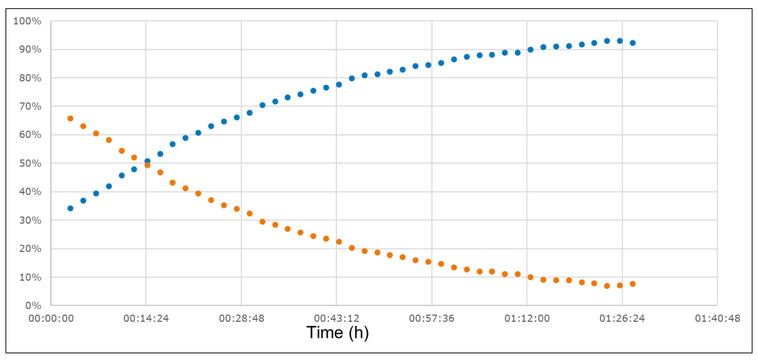


Figure 3. FIA of 1-β-O sNAG incubation showing disappearance of 1-O- form in amber and appearance of other isomers in blue.

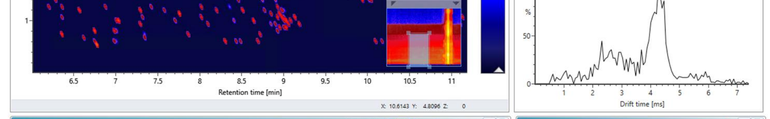


Figure 4. Kinetic plot for FIA of 1-β-O sNAG incubation showing disappearance of starting material with calculation of the half-life

To confirm the assignment of the 1-β-O sNAG and the other isomers, the method development samples were run by LC-MS with 5 passes of the cIM (Figure 5). In the 3D viewer, a significant peak is seen for the 1-O- form in acidic solution whereas in the neutral solution after 2 hrs the other isomers are clearly seen.

Figure 5. LC-MS analysis with 5 passes of the cIM of 1-β-O sNAG in acid (upper plot) and after incubation (lower plot)

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 acyl migration.

References

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