The effect of acidic conditions on the rotamery phenomenon

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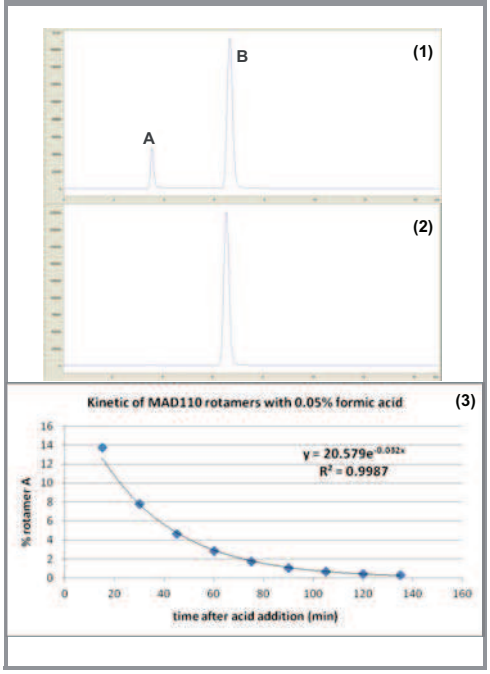
Introduction

The MAD110 molecule has anti-tumoral properties. To perform an in vivo study in mice, a UHPLC/MS method has to be developed for the MAD110 determination in mice serum. However, this molecule presents two major rotamers separated by liquid chromatography and with differences in their mass spectra. Rotamers are conformational isomers (conformers) which differ by a rotation around one single σ bond of the molecule. The rotamery phenomenon must be controlled as it reduces the analysis sensitivity and causes quantification errors. Sample preparation in acidic conditions appears to displace the rotamery equilibrium in favour of one rotamer. To have a better representation of the acid effect, a kinetic study was performed in different conditions.

Experimental conditions

MAD110 was analysed by ultra high pressure liquid chromatography using a Poroshell SB-C18 column (2.1×50mm; 2.7µm) and a methanol/water 47:53 (v/v) mobile phase. The flow rate was regulated at 0.5mL/min. The column temperature was set at 40°C and the run method lasted 15 minutes. The mass spectrometer consisted in an API-electrospray source and a single quadrupole analyser. Acquisition was performed using the Single Ion Monitoring (SIM) mode.
A stock solution (40 µg/mL in methanol) was diluted 20 times in a methanol/water 50:50 (v/v) mixture containing 0.05; 0.1; 0.2 and 0.3% of formic acid respectively. The sample was continuously injected until the minor rotamer quantities were under the limit of detection.

Figure 1: Chromatogram obtained after injection of MAD110 samples without acid.
Figure 2: Chromatogram obtained after injection in acidic conditions.
Figure 3: Evolution of rotamer A proportions as a function of contact time with acid.



Results

When acid was added (at the time of the first injection), two rotamers were present: rotamer A (minor) with a retention time of 3.5min and rotamer B (major) with a retention time of 6.5min (figure 1). When the contact time with the acid increased, rotamer A proportions decreased until rotamer B was the only one detectable conformer (figure 2). Figure 3 shows the exponential decrease of rotamer A proportions. A rise in acid percentages, from 0.05% to 0.3%, speeded up the decline. 2h15, 2h00, 1h15, 45min for 0.05%, 0.1%, 0.2%, 0.3% respectively were necessary to detect the exclusive presence of rotamer B.

Conclusion

Addition of 0.05% to 0.3% of formic acid in MAD110 samples displaces the rotamery equilibrium until only one rotamer is detectable. The proportion of the minor rotamer depends of the contact time with acid and its decline can be accelerated by increasing the acid concentration. So, the rotamery phenomenon of MAD110 molecule can be controlled with sample preparation in acidic conditions. This discovery will be of great interest during the extraction process development.

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