

## Recovery of Codeine-6-Glucuronide with Beta-Glucuronidase

Several customers have reported lower than expected recovery of Codeine-6-glucuronide with beta-glucuronidase.

Some of our customers have resolved the issue and provided suggestions. The problem appears related to substrate specific enzyme reaction rate and/or capacity, with better reaction rate and conversion for morphine-3-glucuronide than opiate-6-glucuronides. They have observed better results using a 4 fold excess of enzyme relative to glucuronide over their normal procedure. This can be achieved either by reducing the concentration/volume of the glucuronide or increasing the concentration/volume of the enzyme. For LC/MS/MS applications lowering the concentration of glucuronide, buffer and IS to 1/4th was recommended due to lower sensitivity of the LC/MS/MS technique relative to GC/MS. Stereochemical position of the glucuronide or activity or type of glucuronidase could be factors that affect reaction rate and recovery.