# ANALYSIS OF ACE INHIBITORS IN CLINICAL SAMPLES – THE STRENGTHS OF ESI-LC-MS/MS AS A TROUBLESHOOTING TOOL

Andrew D de Jager, Linda A. Wright and Debra M. Siebert

*Q-Pharm Pty Limited, 300C Herston Road, Herston, QLD 4029, Australia.*

Acetyl Choline Esterase (ACE) inhibitors are used in the treatment of hypertension and congestive heart failure. Drugs of this class are typically hydrolised to more active *di*-acid forms *in vivo*, as well as other less significant metabolites. Analysis of a range of ACE inhibitors has revealed shortcomings in existing 'pril' analytical literature. Surprisingly high levels of acyl-glucuronide conjugated forms of drug and respective *di*-acids have been identified in clinical samples. Labile glucuronide conjugates have been shown to readily revert back to the parent compound both during sample preparation and in the mass-spectrometer source (so-called CID), serving as a timely reminder that chromatographic resolution remains an important parameter in high throughput LC-MS/MS.

Differences observed between clinical samples and calibrators expose flaws in existing industry method validation practices. While so-called batch quality control samples are intended to 'vouch' for the integrity of unknown samples in an analytical run, they differ from real samples chiefly because they necessarily do not contain metabolites of the drug under investigation.

Matrix stability studies (a mandatory component of bioanalytical method validation) are almost always performed on samples prepared *ex-vivo*. There exists, in certain instances, the very real possibility that while quality controls samples demonstrate acceptable stability, the same is simply not true for real 'patient' samples, primarily owing to the absence of metabolites. In recent times, industry heavyweights have begun to make noises regarding method validity on so-called 'clinically incurred' samples.

Initially, a generic 'pril' assay method was developed which involved acidic derivatization. While the method passed all industry validation requirements (performed on *ex-vivo* calibrators), analysis of data revealed that while post-validation batches were comfortably meeting acceptance criteria, not all was well with the clinical samples. Further investigation revealed that the co-presence of high levels of labile metabolites (barely touched upon in analytical literature) was the culprit, requiring a complete rework of the analytical method.

For many clinical trial units, obtaining live samples for method validation presents a significant ethical problem. Q-Pharm has developed an imperfect, yet sensible approach to collecting clinically incurred samples for assessing the validity of assay methods when used on real samples.

The 'pril' journey represents a fascinating trouble-shooting exercise in which the humble triple-quad mass spectrometer proved to be an invaluable tool in firstly identifying and solving this complex analytical problem. Moreover, it is clear that in the ever-changing landscape of what regulators require for method validation, the limitations of what *ex-vivo* calibrators do and do not 'prove' regarding real samples needs to be well understood.