A Problem of Too Many Pills and the Arcane World of Dihydrocodeine

**Background**

Dihydrocodeine (DHC) holds analytical interest both as an active opiate metabolite and as a pharmaceutical agent. As one of the last remaining Schedule III semisynthetic opiates, interest in DHC may increase. In addition to being a pharmaceutical, DHC is also metabolically formed from the 6-keto reduction of hydrocodeone (HC). However, the enzymatic reduction of HC produces two stereoisomers; DHC with a hydroxyl group in the 6-beta configuration, and isodihydrocodeine (IDHC) with the hydroxyl group in the 6-alpha position (Figure 1). The relationship between DHC and IDHC is not well described in literature. The analysis of DHC is further complicated in IDHC with the hydroxyl group in the 6-position (Figure 4).

**Methods**

The extraction methodology utilizes a two step solvent (acetonitrile/methanol) dilution with a second aqueous dilution-based extraction of urine specimens followed by UPLC-MS/MS analysis on an Acquity® UPLC (Waters Technologies Corp.) connected to 5500® Triple Quadrupole Mass Spectrometer (Sciex). UPLC parameters are presented in Table 1 and monitored transitions are presented in Table 2.

The methodology quantitatively measures DHC over the linear range of 20.0 – 2,000.0 ng/mL with qualitative assessment of IDHC, codeine, HC, oxymorphone, and noroxycodone to aid in the interpretation of results.

The validated analytical method was utilized to evaluate 49 HC and 4 DHC pain management patients.

**Results**

- Quantitative analysis of DHC was fully validated in human urine across a linear range of 20.0 – 2,000.0 ng/mL.
- The metabolism of HC was found to favor IDHC over DHC in the 49 HC users evaluated (Figure 3).
- The presence of IDHC can successfully be utilized to discriminate the source of DHC as either from DHC administration or HC metabolism (Figure 3 insert).
- Using real samples, the enzymatic hydrolysis step of DHC-glucuronide during sample extraction was found to occur at a much slower rate than other related opiates. The enzymatic hydrolysis of IDHC-glucuronide required 30 minutes versus 120 minutes required by DHC to achieve 80% hydrolysis efficiency (Figure 5).
- Slow chromatographic conditions were required to resolve DHC and IDHC from other opiates. High levels of codeine can overlap with the transitions of DHC and IDHC due to the presence of the 13C2-codeine isotope. Complete baseline resolution between DHC and IDHC from 13C2-codeine interference was not achievable (Figure 6a).
- Other opiates with overlapping transitions were more easily resolved (Figure 6b) with the exception of noroxycodone (Figure 6c) which was not resolvable from IDHC. However, noroxycodone could be distinguished from IDHC through a single, unique MRM transition 302.2 > 245.2 m/z.

**Conclusions**

Analysis of DHC is significantly complicated from combinations of other administered opiates and metabolites interfering with accurate quantitation for patients. Poor hydrolysis efficiency should be considered during analysis. Additional information can be gleaned from the presence or absence of IDHC as a qualitative indicator of HC metabolism. Accurate DHC quantitation is achievable when cognizant of all aspects of metabolism and co-morphology within the opiate drug class.

**References**