Identification and Separation of Opioid Metabolites in Urine by LC-MS/MS

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INTRODUCTION

With the recent addition of oxycodone, oxymorphone, hydrocodone and hydromorphone to the October 2017 Mandatory Guidelines for Federal Workplace Drug Testing Programs, it is imperative that samples containing such drugs are accurately tested by laboratories. Chromatographic separations are essential for the analysis and correct identification of opioids by LC-MS/MS as some of the compounds are structural isomers and opioid metabolites can form the same fragments in the mass spectrometer. In particular, our lab found that samples containing oxycodone and oxymorphone have interfering peaks detected by LC-MS/MS, which can make accurate quantitation of codeine, morphine and hydromorphone challenging.
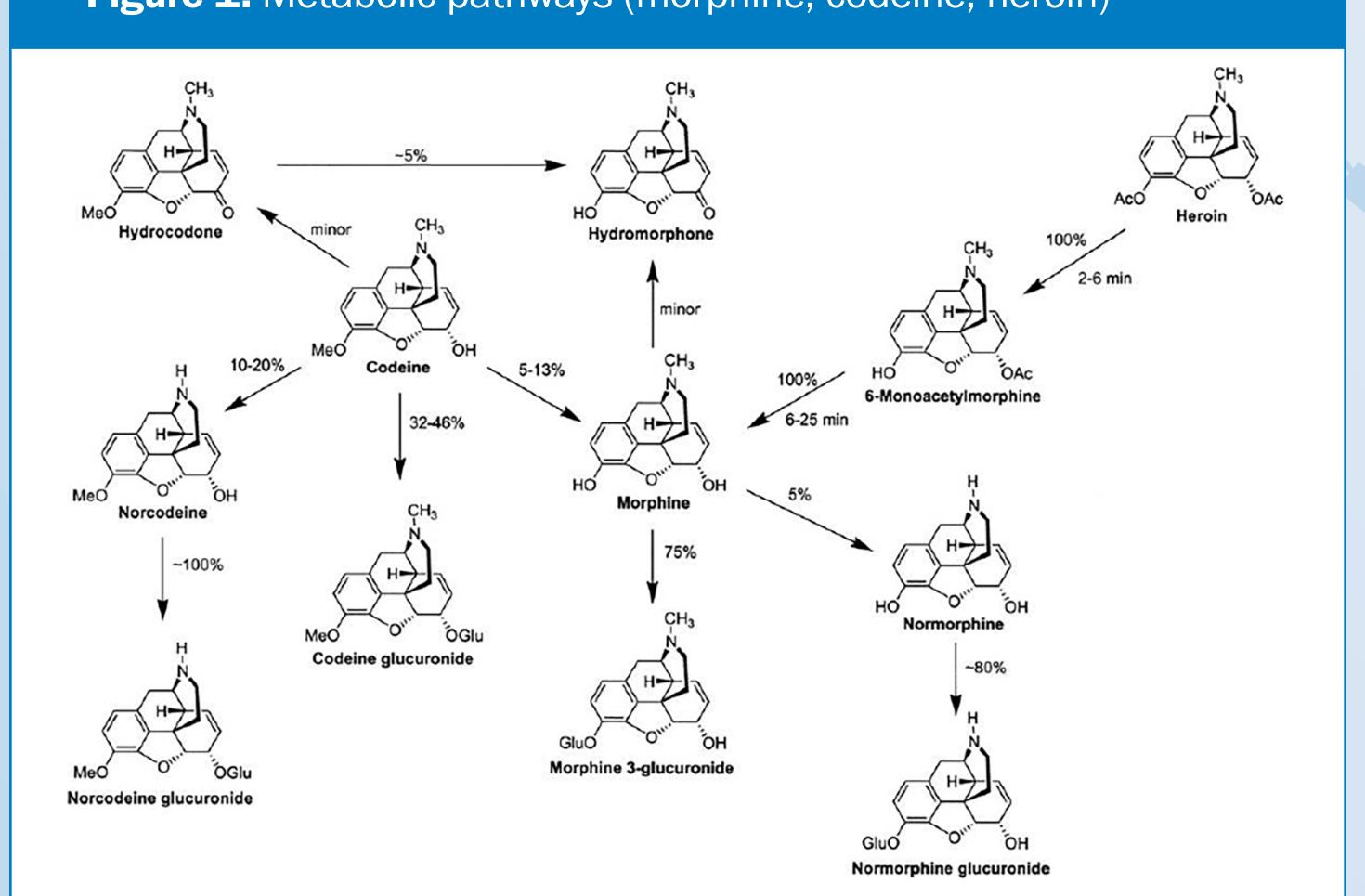
OBJECTIVE

To identify opioid metabolites found in patient samples that can lead to possible misidentifications of codeine, morphine, hydrocodone, hydromorphone and dihydrocodeine by LC-MS/MS analysis.

METHOD

Fifteen different opioid metabolites were obtained: 6α-oxycodol, 6β-oxycodol, 6α-oxymorphol, 6β-oxymorphol, nor-6α-oxycodol, nor-6β-oxycodol, 6α-oxycodol N-oxide, 6β-oxycodol N-oxide, 6β-hydrocodol and 6β-hydromorphol (Cayman Chemical), and norcodeine, noroxycodone, norhydrocodone, noroxymorphone and 6-AM (Cerilliant). All analytes were diluted to 1,000ng/mL except for norcodeine and norhydrocodone, which were diluted to 5,000ng/mL in negative human urine and negative human urine spiked at 40% of the NLCP guidelines cutoff for morphine, hydromorphone, dihydrocodeine, codeine and hydrocodone. Samples were then prepared for analysis using enzymatic hydrolysis and injected onto a Shimadzu Nexera UHPLC system equipped with a Restek Raptor Biphenyl column. Detection was performed using a Sciex API6500 QTRAP with electrospray ionization in positive mode. Analytical separations were optimized using different columns of varying stationary phases and lengths.
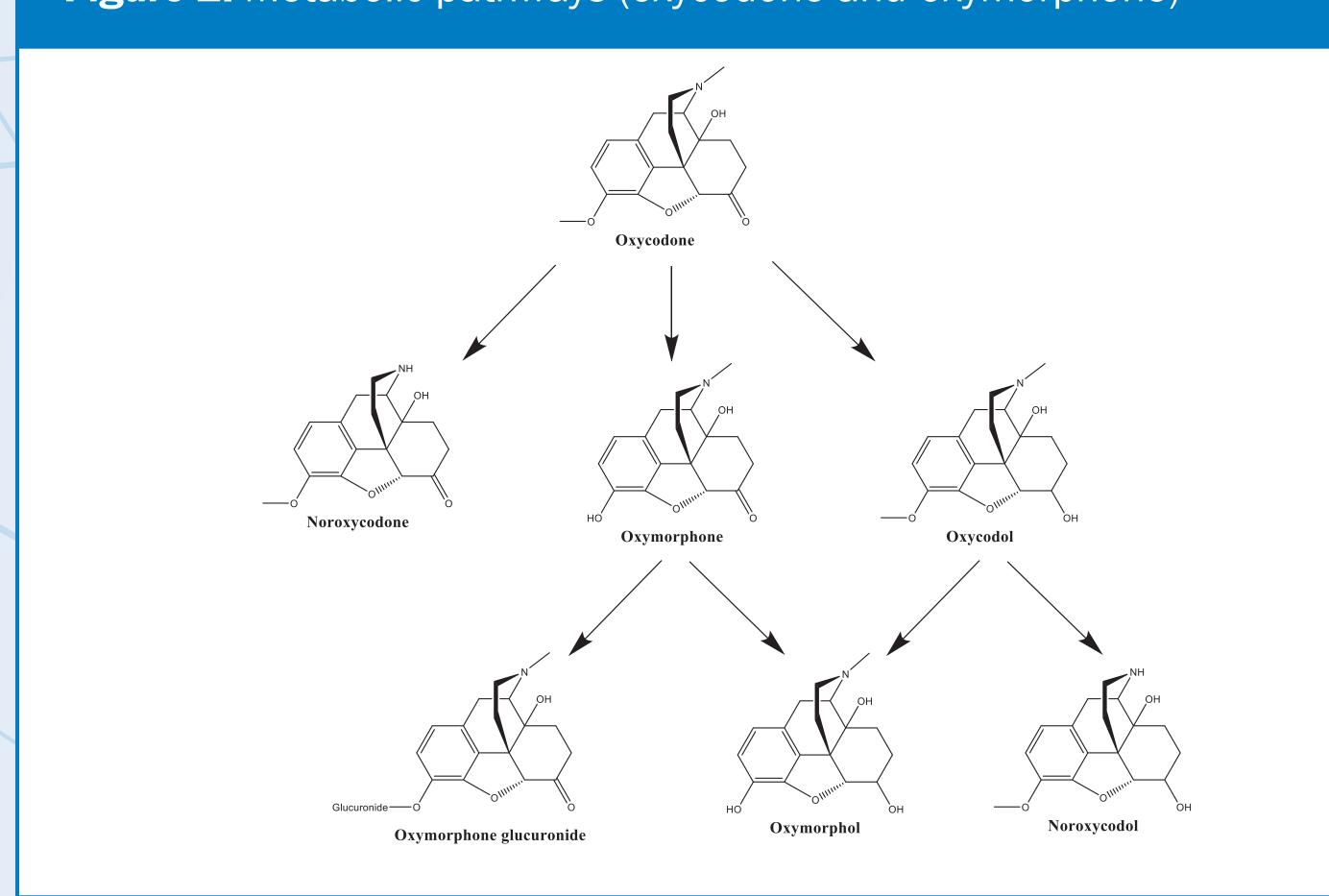
Figure 1. Metabolic pathways (morphine, codeine, heroin)
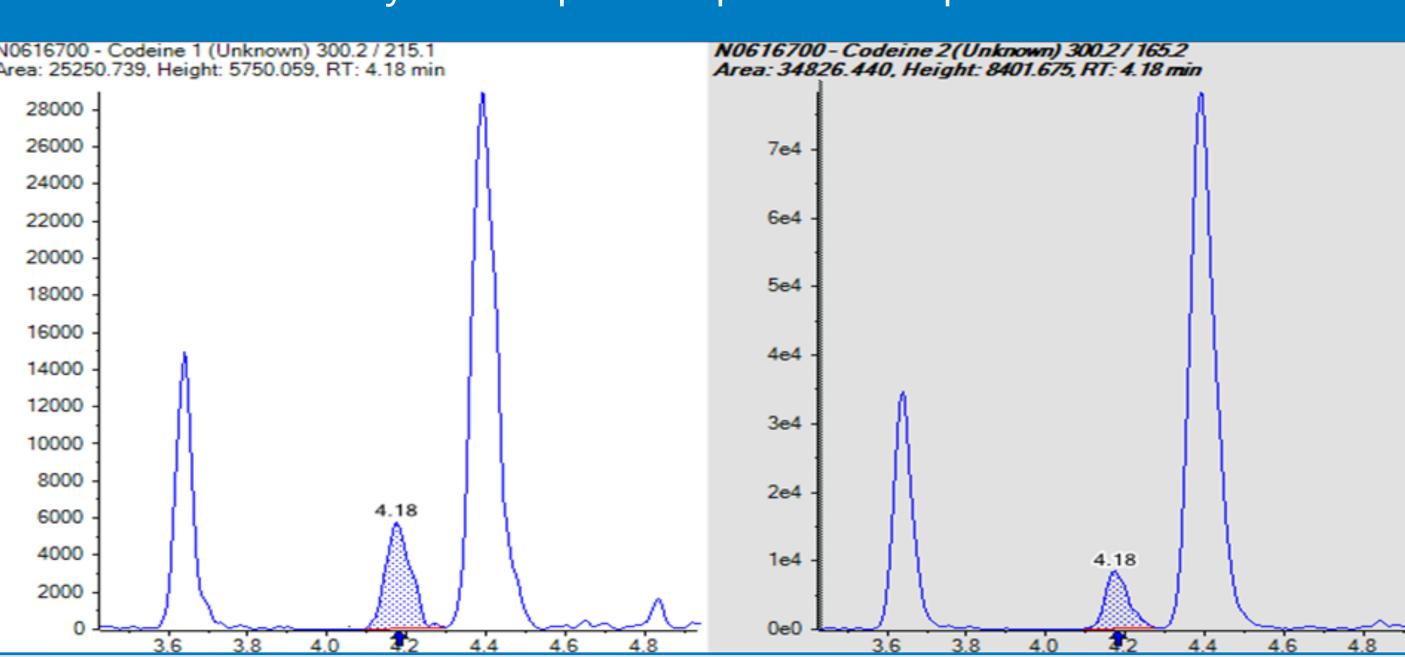
RESULTS AND DISCUSSION

Most opioids have the same base chemical structure and vary by slight functional group changes. Due to these structural similarities, they form metabolites that can vary by as little as one mass unit. Metabolites can be seen in the mass windows of the primary drugs of interest when monitored by LC-MS/MS, giving rise to the possibility for misidentification. One way to address this concern is to evaluate known possible interfering metabolites and resolve them chromatographically. See Figures 1 and 2 for metabolic pathways and metabolite structures of common opioids.
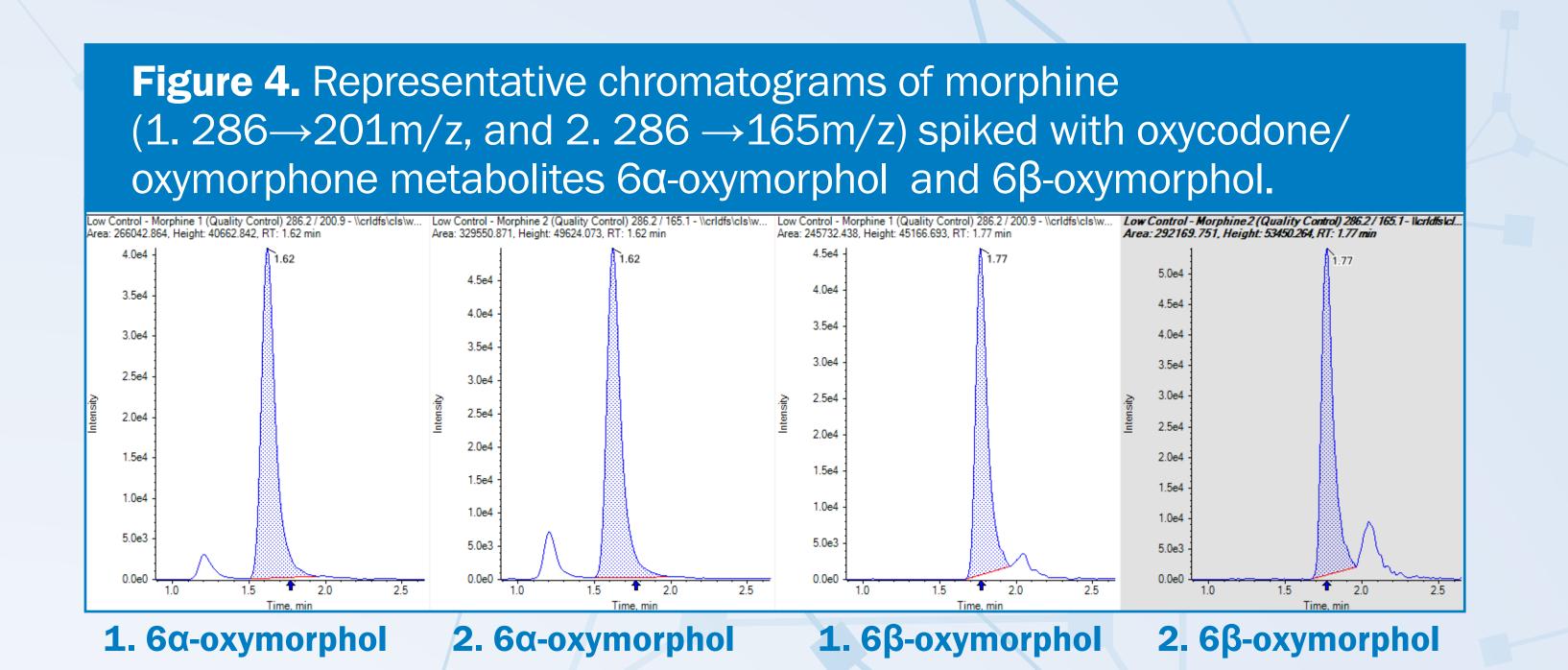
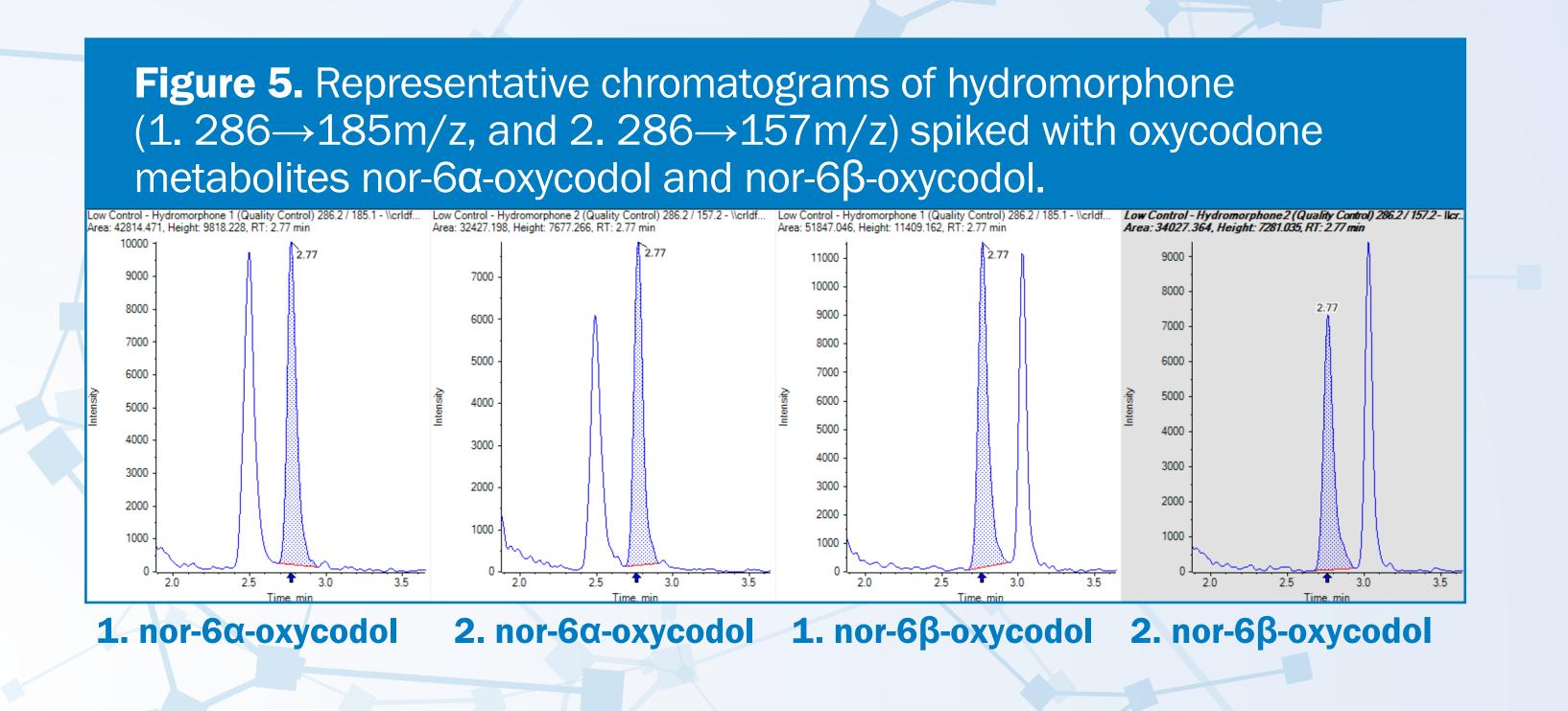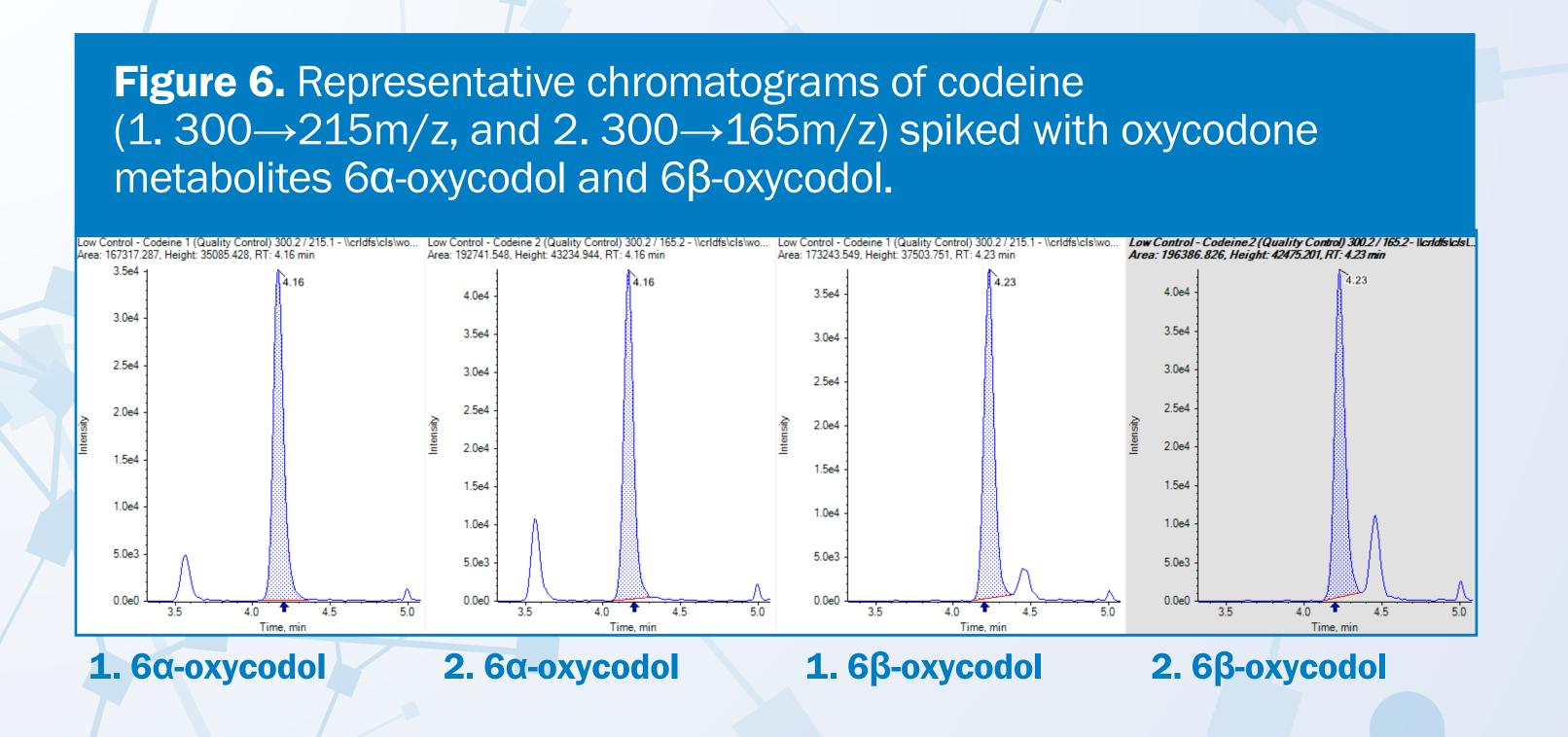**Figure 2.** Metabolic pathways (oxycodone and Oxymorphone)

While testing patient specimens from opioid users during validation, it was observed that samples positive for oxycodone would exhibit doublet or triplicate peaks in the transitions being monitored for codeine. These peaks were not always baseline resolved and would fail ion ratio acceptability criteria, making it challenging to accurately quantitate codeine. In Figure 3, for example, a small amount of codeine and two unidentified oxycodone metabolites are observed in a sample positive for oxycodone. Samples spiked individually with fifteen different metabolite standards were used to determine the identity of the unknown metabolites. Example chromatograms are shown in Figures 4-8.
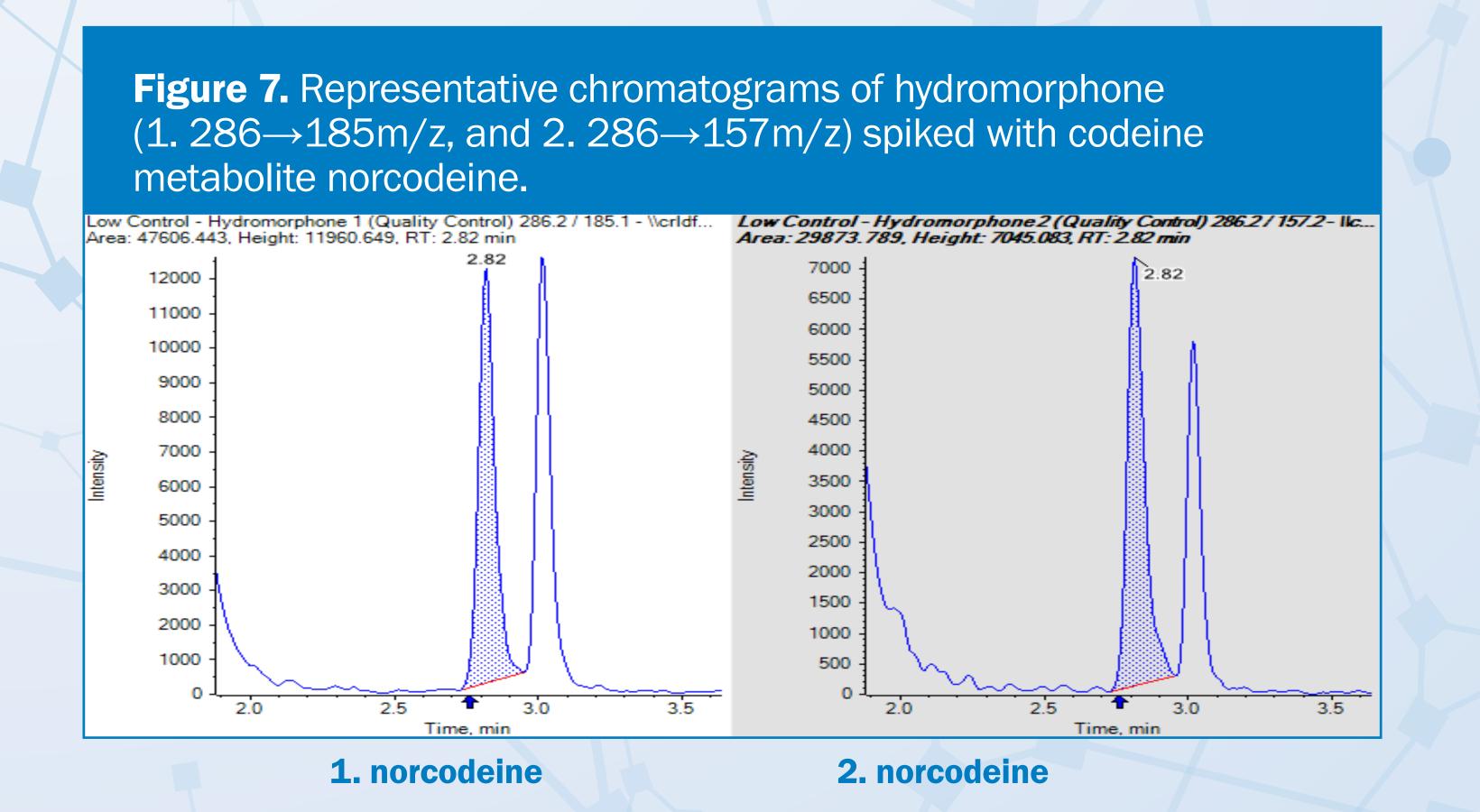
Figure 3. Representative chromatograms of codeine with unknown interferences in oxycodone positive patient sample.

Figure 4. Representative chromatograms of morphine (1. 286→201m/z, and 2. 286→165m/z) spiked with oxycodone/oxymorphone metabolites 6α-oxymorphol and 6β-oxymorphol.

1. 6α-oxymorphol   2. 6α-oxymorphol   1. 6β-oxymorphol   2. 6β-oxymorphol

Figure 5. Representative chromatograms of hydromorphone (1. 286→185m/z, and 2. 286→157m/z) spiked with oxycodone metabolites nor-6α-oxycodol and nor-6β-oxycodol.

1. nor-6α-oxycodol   2. nor-6α-oxycodol   1. nor-6β-oxycodol   2. nor-6β-oxycodol

Figure 6. Representative chromatograms of codeine (1. 300→215m/z, and 2. 300→165m/z) spiked with oxycodone metabolites 6α-oxycodol and 6β-oxycodol.

1. 6α-oxycodol   2. 6α-oxycodol   1. 6β-oxycodol   2. 6β-oxycodol

Figure 7. Representative chromatograms of hydromorphone (1. 286→185m/z, and 2. 286→157m/z) spiked with codeine metabolite norcodeine.

1. norcodeine   2. norcodeine

Figure 8. Representative chromatograms of dihydrocodeine (1. 302→201m/z, and 2. 302→171m/z) spiked with hydrocodone metabolite 6β-hydrocodol.
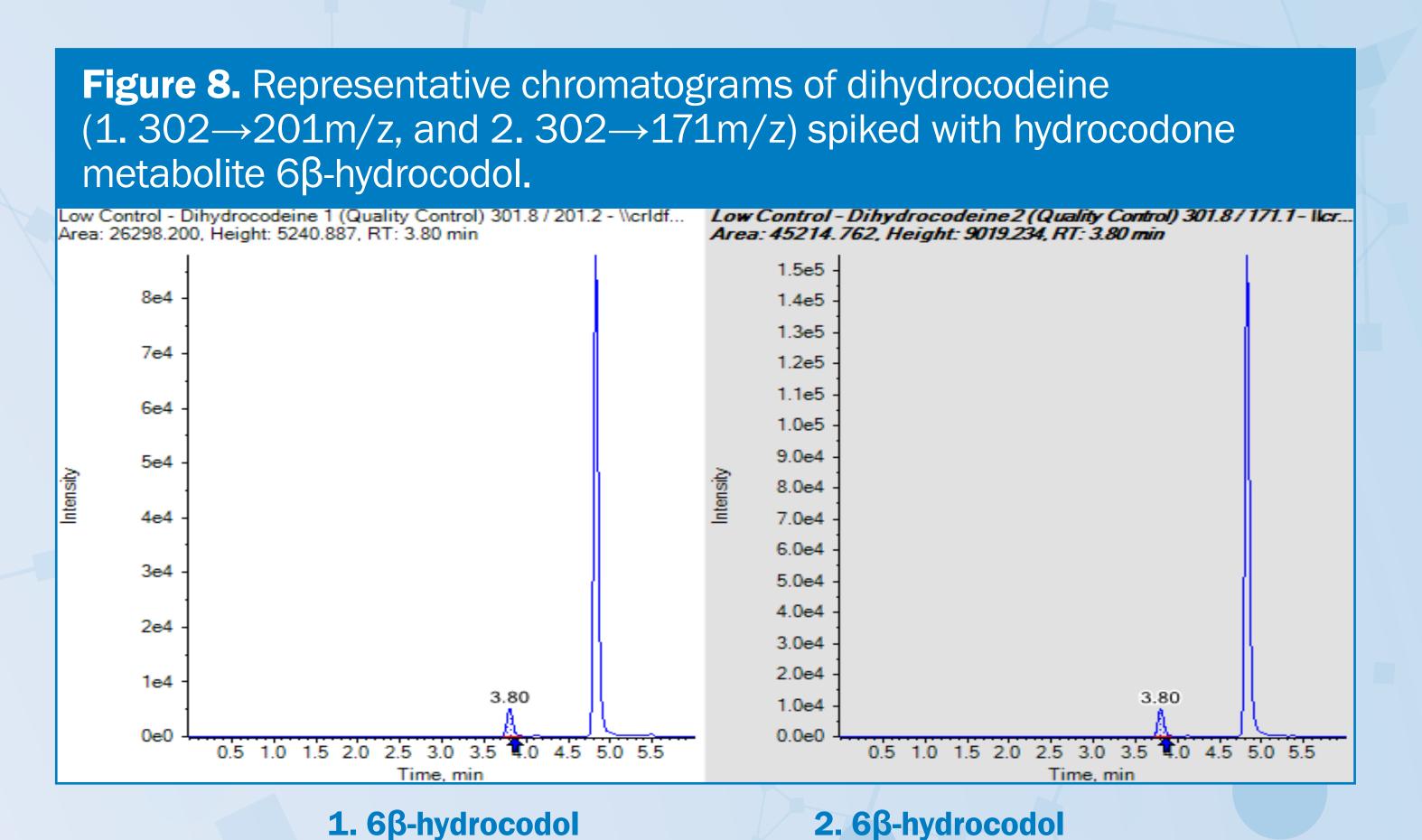
1. 6β-hydrocodol   2. 6β-hydrocodol

All metabolites were baseline resolved from analytes of interest using a 100mm biphenyl column. The previously unidentified oxycodone metabolites observed in patient samples (Figure 3) were determined to be 6α-oxycodol and 6β-oxycodol (Figure 6), which elute near codeine and form fragments with similar ion ratios. All other columns evaluated were not able to fully resolve 6α-oxycodol and 6β-oxycodol from codeine.

CONCLUSION

The analysis of opioids and their metabolites by LC-MS/MS can be challenging in routine human samples due to the complex drug metabolism that occurs. For example, oxycodone metabolizes to noroxycodone (<47%), oxymorphone (11%), oxycodol (8%) and less than 10% excreted as unchanged oxycodone. When testing for opioids, we found that best practice was to use longer columns and run a slower gradient. Additionally, by chromatographically separating metabolites that interfere with analytes of interest, a more robust method was developed that minimized the potential for misidentification or misquantitation.

REFERENCES

Reisfield GM, Salazar E, Bertholf RL. Rational use and interpretation of urine drug testing in chronic opioid therapy. Ann Clin Lab Sci 2007;37(4):301–14. Federal Register. (2017) Mandatory Guidelines for Federal Workplace Drug Testing Programs https://www.samhsa.gov/sites/default/files/workplace/frn_vol_82_7920_.pdf (accessed August, 2018).

DISCLOSURE

No relevant financial or nonfinancial relationships to disclose

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