Morphine and Oxycodone Co-positivity in Pain Management Urine Drug Testing

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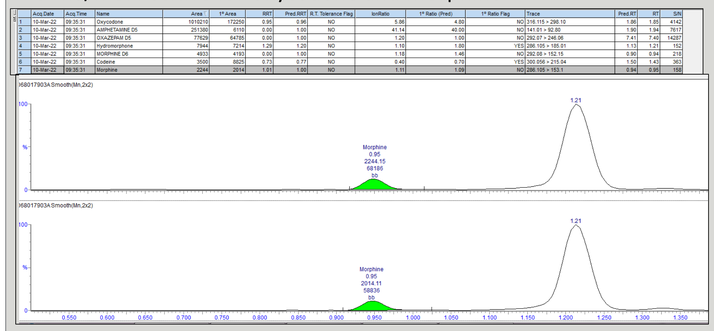
Introduction

Direct LC-MS/MS analysis is preferred for pain management urine drug testing (PM-UDT) . These methods often utilize low cutoffs for positivity to account for variable urine concentration, dosing, and other factors that influence drug excretion. While low detection limits decrease the likelihood of false negative results, the instrument settings required for sensitive analysis may promote the formation of isobaric interferences secondary to in-source fragmentation and/or neutral losses. In particular, these phenomena have the potential to cause false positive results when targeted compounds have structural similarity and/or minor metabolites that are not routinely monitored, as is the case for some opioids in our PM-UDT. In this poster, we present rare occurrences of morphine and oxycodone co-positivity in individuals prescribed oxycodone only. The qualitative method described includes direct LC-MS/MS analysis of 14 compounds and a standard screen then confirm approach for other drug classes. Discussion of co-positive results with PM providers indicate low suspicion for illicit morphine use. This phenomenon is complicated by the fact that some individuals are prescribed multiple opioids concurrently. Literature review suggests that minor metabolites of oxycodone may undergo in-source loss of water to produce morphine isobars in the context of elevated source voltages[1].

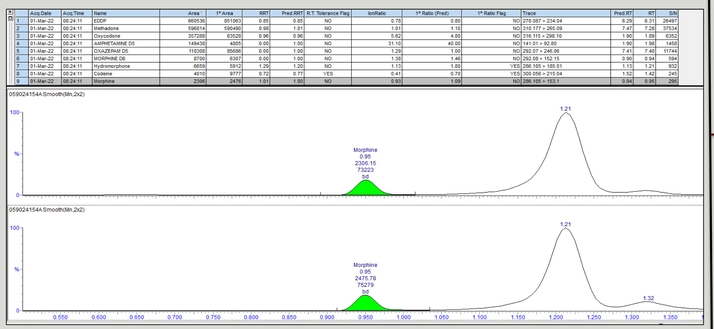
Opioid LC-MS/MS Method

- Dilute and shoot (no hydrolysis): 1:1 mix urine + internal standard mix, 5mcL injection volume
- Waters Acquity UPLC, 2.1x150mm, 1.8µM C18 column
- 8.5 min linear gradient from 87% MPA to 50% MPA, column temp = 50^{o}C
- Waters Xevo TQD, MRM acquisition, positive mode
- Criteria for positivity: RRT, precursor m/z, presence of ≥2 product ions, quantitative ratio of the two most abundant product ions, and signal of the two most abundant product ions ≥ compound specific LOD
- Cutoffs: 25 ng/mL with the following exceptions: 6-AM & naloxone (10ng/mL), buprenorphine (5 ng/mL)

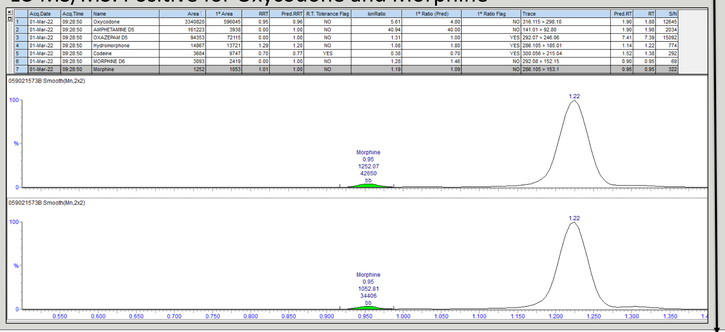
Pt 1: Prescribed Oxycodone and Morphine
IA Screen: Positive for Oxycodone and Opioids
LC-MS/MS: Positive for Oxycodone and Morphine



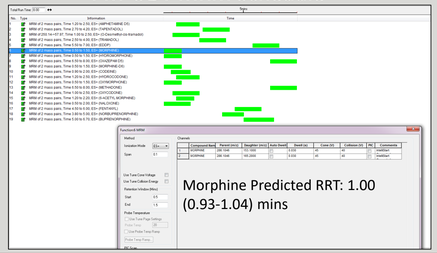
Pt 2: Prescribed Oxycodone only
IA Screen: Positive for Oxycodone only
LC-MS/MS: Positive for Oxycodone and Morphine



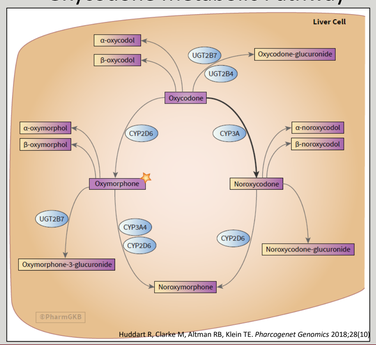
Pt 3: Prescribed Oxycodone only
IA Screen: Positive for Oxycodone only
LC-MS/MS: Positive for Oxycodone and Morphine



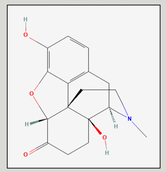
PM-UDT MRM Schedule



Oxycodone Metabolic Pathway

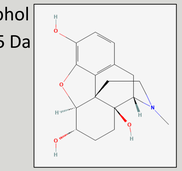


Possible Mechanism of Interference[1]



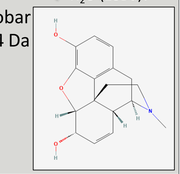
Oxymorphone
MW: 301.34 Da

6-keto reduction (in-vivo)



α/β-Oxymorphol
MW: 303.35 Da

In-source loss of H_2O (18Da)?



Morphine Isobar
MW: 285.34 Da

Details/Troubleshooting

- Verified correct instrument/analysis settings; phenomenon occurs on multiple instruments but only the PM-UDT method

-Cannot rule-out a method-specific problem, but this is not an instrument-specific issue.

- Sent out suspect specimens to a reference lab with identical cutoffs. Results for morphine were reported as either "interfering substance" or negative.

-Likely related to both biological and analytical issues

- Spiked Oxycodone standard into negative urine at high concentrations, did not replicate problem (clean morphine EIC)

-Suggests biological interference, does not rule-out analytical problem

- Separated Morphine and Oxycodone MRM functions in time, without resolution

-Suggests related to biological interference caused by a substance that co-elutes with morphine

- Review of literature indicates minor Oxycodone metabolites may form a morphine isobar after in-source loss of water[1]

Conclusions

- Rare cases of co-positivity for morphine and oxycodone, from individuals taking oxycodone only, may be caused by both biological (minor oxycodone metabolites) and analytical sources of error (source settings)
- Temporary solution: Any PM-UDT specimen that is co-positive for oxycodone and morphine by LC-MS/MS requires a positive opioid immunoassay (300 ng/mL) in order to report morphine
- Future studies/other solutions: Test a standard for α/β-oxymorphol, try alternative morphine transitions, monitor for morphine metabolites (glucuronides), modify chromatographic gradient, modify sensitivity of method for morphine

Reference

1. Munoz-Munoz AC, Pekol T, Schubring D, Johnson C, Andrade L. Identification of Novel Opioid Interferences using High-Resolution Mass Spectrometry. *J. Anal Tox*. 2018;42: 6-16.