TECHNICAL NOTE

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A summary of "Isomer interferences observed during the development of a 47-analyte HRAM LC-MS/MS method for urine drug testing"

Overview:

The rise in the use of opioids for pain management and illicit use has increased the need for urine drug testing laboratories to monitor a wide range of opioid analytes. Detection and confirmation of drug analytes in urine is typically done with a preliminary immunoassay and subsequent analysis using GC/MS or LC/MS. Liquid chromatography coupled with quadrupole mass spectrometers and high resolution accurate mass (HRAM) instrumentation is becoming more prevalent for confirmation of drug analytes due to its improved specificity, shorter run times, simpler sample preparation, and lower detection limits. However, laboratories must be aware that this instrumentation may not be able to separate interferences arising from isomeric metabolites that are not typically monitored. The use of a non-selective -glucuronidase may increase the detection levels of these metabolites. The presence of these interferences was discovered during the development of a

Materials and Methods:

ethod and these interferences are reported here.

75 µL of patient urine sample was combined with 300 µL of master mix containing internal standards and IMCSzyme®. Samples were incubated at 65°C for 60 minutes and centrifuged at 4000 rpm for 7 minutes before analysis on a Waters ACQUITY UPLC® I-Class coupled with a Thermo Scientific Q-Exactive Orbitrap™.

Results:

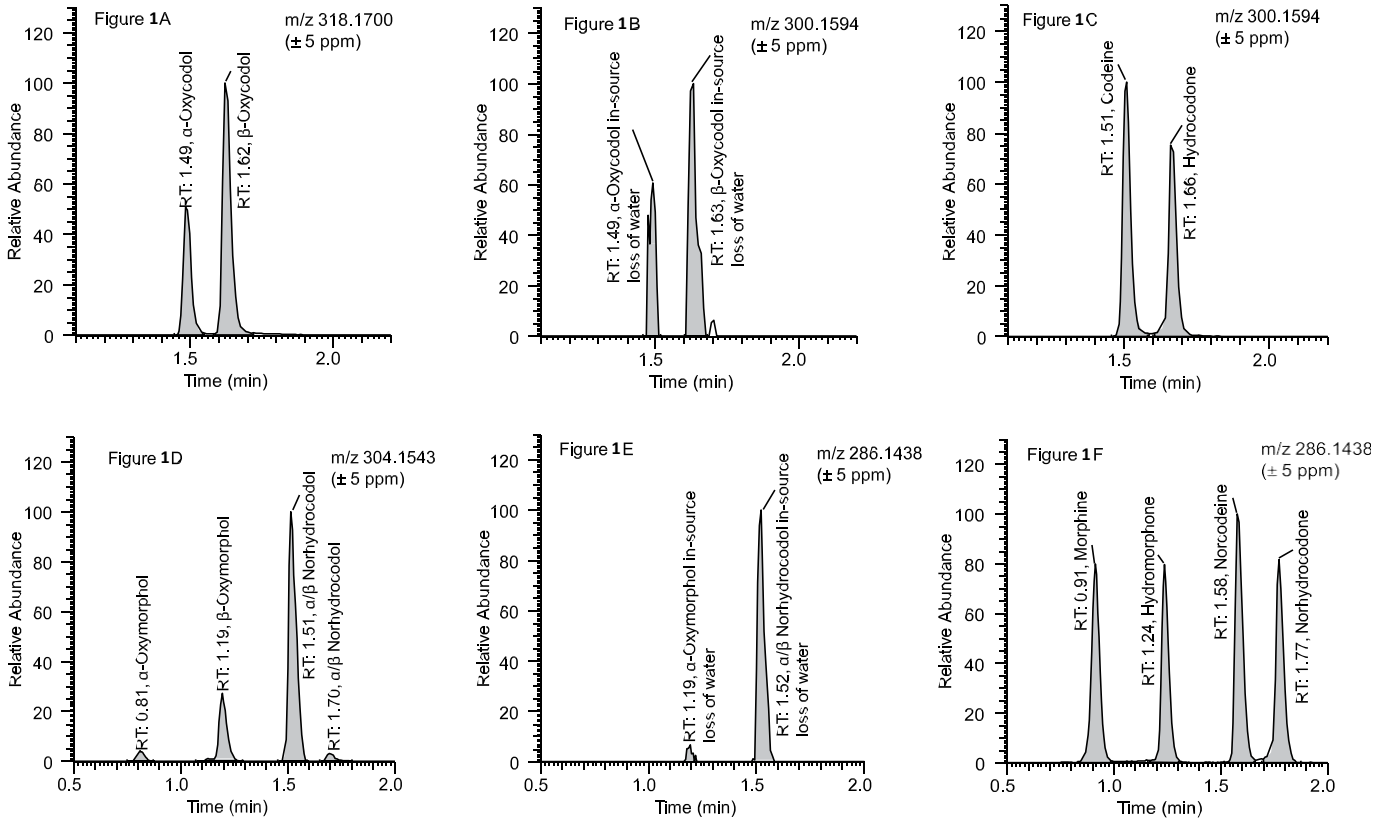

Figure 1. LC-MS/MS Chromatograms of (A) α/β-Oxycodol in a patient specimen positive for Oxycodone; (B) -Oxycodol and -Oxycodol loss of water due to in-source fragmentation; (C) Codeine and Hydrocodone in a standard sample (D) α/β-Oxymorphol and α/β-Noroxycodol in a patient specimen positive for Oxycodone; (E) -Oxymorphol and or -Noroxycodol loss of water due to in-source fragmentation; (F) Morphine, Hydromorphone, Norcodeine and Norhydrocodone in a standard sample.

This information was summarized by IMCS from the technical poster "Isomer interferences observed during the development of a 47-analyte HRAM LC-MS/MS method for urine drug testing" presented by Ana Grenier Dominion Diagnostics at MSACL 2017

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Results Continued:

Table 1. Potential Analyte Interferences

Analyte	Precursor m/z(H+)	Formula (H+)	Interference	Interference m/z(H+)	Formula (H+)	Source of Interference	Interference loss of water (H+)	Formula loss of water (H+)	Reporting Outcome
Codeine	300.1594	C18H22NO3	Oxycodone metabolites, α/β-oxycodol	318.1700	C18H24NO4	In-source loss of water produces isomeric precursor and product ions	300.1594	C18H22NO3	Over-reporting or false positive for codeine
Hydrocodone	300.1594	C18H22NO3	Oxycodone metabolites, α/β-oxycodol	318.1700	C18H24NO4	In-source loss of water pro- duces isomeric precursor and product ions	300.1594	C18H22NO3	Over-reporting or false positive for hydrocodone
Norcodeine	286.1438	C17H20NO3	Oxycodone metabolites, α/β-noroxycodol	304.1543	C17H22NO4	In-source loss of water produces isomeric precursor and product ions	286.1438	C18H22NO3	Over-reporting or false positive for norcodeine
Norhydrocodone	286.1438	C17H20NO3	Oxycodone metabolites, α/β-noroxycodol	304.1543	C17H22NO4	In-source loss of water produces isomeric precursor and product ions	286.1438	C18H22NO3	Over-reporting or false positive for norhydrocodone
Hydromorphone	286.1438	C17H20NO3	Oxycodone metabolites, α/β-oxymorphol	304.1543	C17H22NO4	In-source loss of water pro- duces isomeric precursor and product ions	286.1438	C18H22NO3	Over-reporting or false positive for hydromorphone
Morphine	286.1438	C17H20NO3	Oxycodone metabolites, α/β-oxymorphol	304.1543	C17H22NO4	In-source loss of water produces isomeric precursor and product ions	286.1438	C18H22NO3	Over-reporting or false positive for morphine
Oxycodone	316.1543	C18H22NO4	Hydrocodone metabolite, hydrocodone N-oxide	316.1543	C18H22NO4	Presumptive hydrocodone metabolite	N/A		Over-reporting or false positive for oxycodone
Oxymorphone	302.1387	C18H22NO4	morphine metabolite/ impurity, morphine N-oxide	302.1387	C17H20NO4	Presumptive hydrocodone metabolite	N/A		Over-reporting or false positive for oxymorphone
Noroxycodone	302.1387	C18H22NO4	Hydrocodone metabolite, N-hydroxynor-hydrocodone	302.1387	C17H20NO4	Presumptive hydrocodone metabolite	N/A		Over-reporting or false positive for noroxycodone

Conclusions:

The presence of isomeric metabolites not typically monitored in patient urine samples is something urine drug testing labs must be aware of to avoid over-reporting or obtaining false positives. Because these metabolites are present only in patient samples, external controls are not a good indicator that a method is free of interference. This work provides a summary of some of the potential opioid interferences in an effort to raise awareness of the importance of running patient samples early on in the process of method development.

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