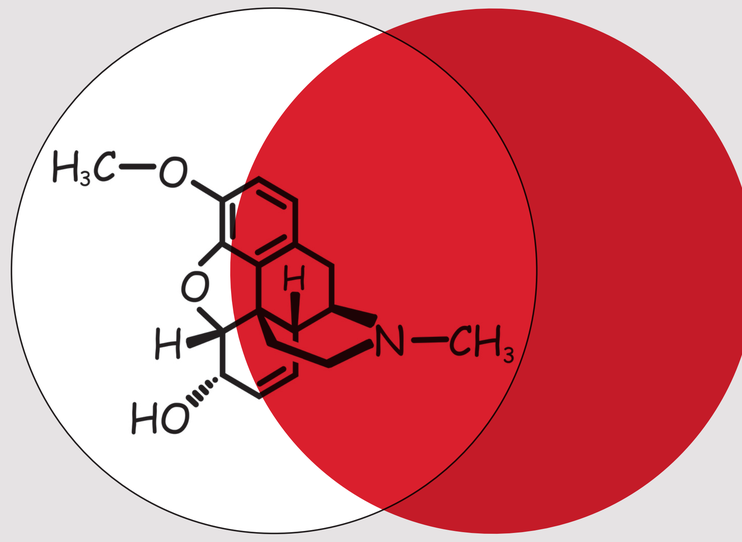
Investigation of a false positive codeine interference in an AACB/RCPAQAP Urine Toxicology Survey sample



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Introduction

A false positive codeine interference was suspected following review of AACB/RCPAQAP Urine Toxicology survey result - Cycle 29 – Sample 2. Although 27% of respondents reported the detection of codeine (median recovery 109 µg/L) using LC-MS based techniques, the high percentage of laboratories failing to detect codeine prompted an investigation into the presence of a potential interference. Several laboratories also observed that the closely eluting interference produced identical product ions for codeine, though ion ratios were not consistent. A participant also reported a similar interference in samples containing high concentrations of oxycodone using a LC-MS/MS technique. An investigation was subsequently undertaken using LC-QTOF-MS to identify the interfering substance.

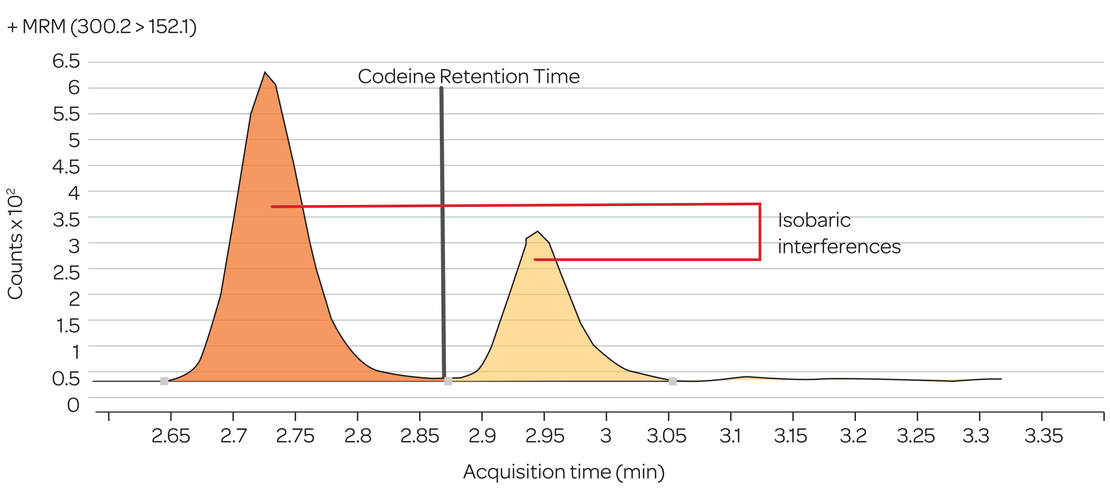

Figure 1a. Codeine interference – Laboratory A

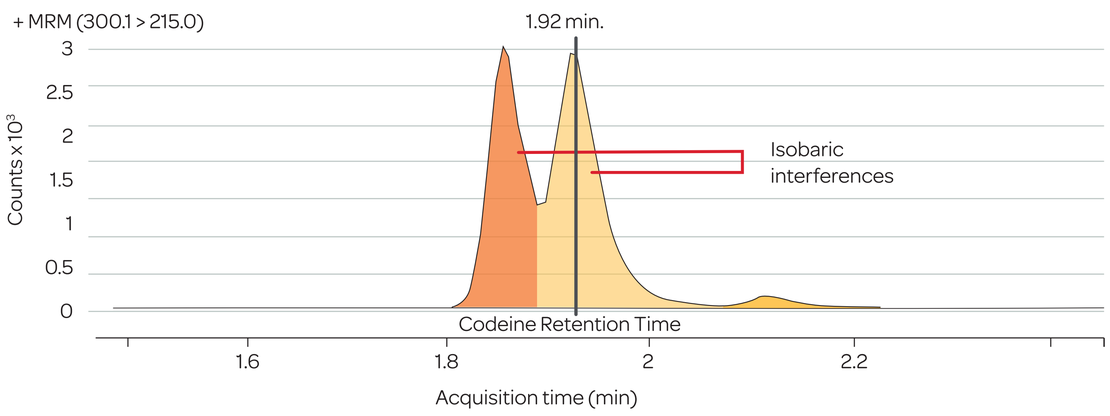

Figure 1b. Codeine interference – Laboratory B

Table 1. Interference Ion Ratio

Laboratory	Product Ions	Interference Ion Ratio	Codeine Ion Ratio
A	152.1, 165.1, 215.0	19.1 (Fail)	84.7
B	165.0, 215.0	132.7 (Fail)	87.4

Method

Sample data obtained by data-independent acquisition (Waters Xevo G2-XS QTOF) for survey 29-02 was interrogated using QTOF software elucidation tools (UNIFI Software version 1.9.3). Presumptive identification of the suspected interference was achieved using elemental formula prediction based on accurate mass and concurrently obtained isotopic information. Identification was further supported by the use of *in-silico* fragmentation to theoretically fragment a proposed molecular structure and match predicted fragments with product ions acquired in the analysis.

Results

Review of untargeted QTOF-MS data from Survey 29-02 confirmed the presence of oxycodone and associated metabolites. As a metabolite of oxycodone was suspected as a candidate for the interference, additional compounds acquired at an identical retention time to codeine were reviewed. A prominent signal was also observed at $[M+H]^+$ *m/z* 318.1695 co-eluting with the suspected codeine interference. Using QTOF-MS elucidation tools, the molecular formula $C_{18}H_{23}NO_4$ for the unknown compound was proposed utilising accurate mass and concurrently acquired isotopic data. Utilising automatic interrogation of the Chemspider.com database, the compound was presumptively identified as 6-oxycodol. 6-oxycodol is a minor metabolite of oxycodone, consisting of two isomers 6-α-oxycodol and 6-β-oxycodol that are excreted in urine in both free and conjugated forms.[1] Further analysis utilising *in-silico* fragmentation tools, was able to identify 40 product ions in the acquired data that were proposed to originate from the 6-oxycodol molecular structure. Also identified was the formation of a water loss fragment of 6-oxycodol that was isobaric with codeine (See Figure 2). Formation of this 6-oxycodol fragment was also proposed to occur in the mass spectrometer ion source.

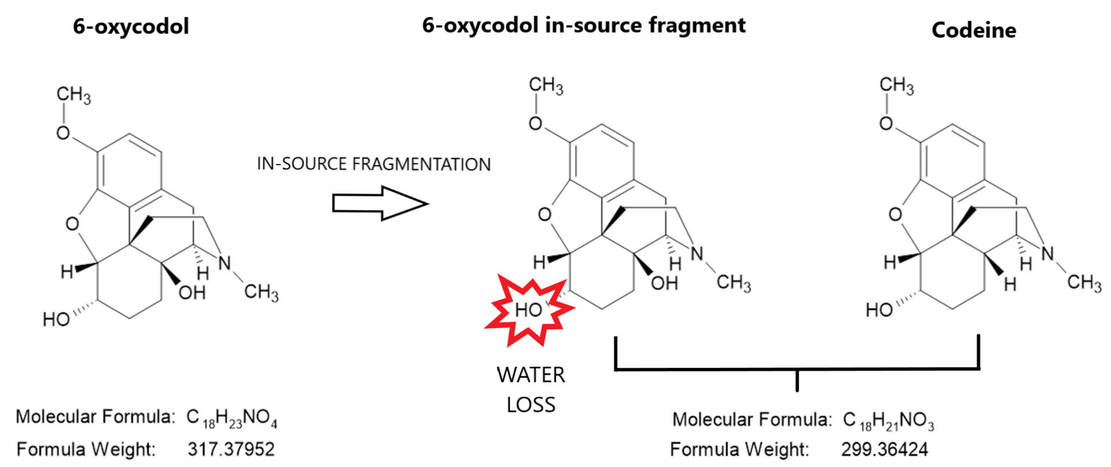

Figure 2. Formation of 6-oxycodol in-source fragment.

To confirm the identity of the interference as 6-oxycodol, certified reference material for both 6-α-oxycodol and 6-β-oxycodol were sourced. Analysis of 6-α-oxycodol certified reference material by the LC-QTOF-MS method confirmed the formation of a water loss fragment isobaric with codeine. Also generated were the expected product ions required for the identification of codeine in this method. The retention time of 6-α-oxycodol was consistent with the first eluting inference peak (See Figure 3). It was suspected in this method that the later eluting peak corresponded to 6-β-oxycodol which would be confirmed with certified reference material when available.

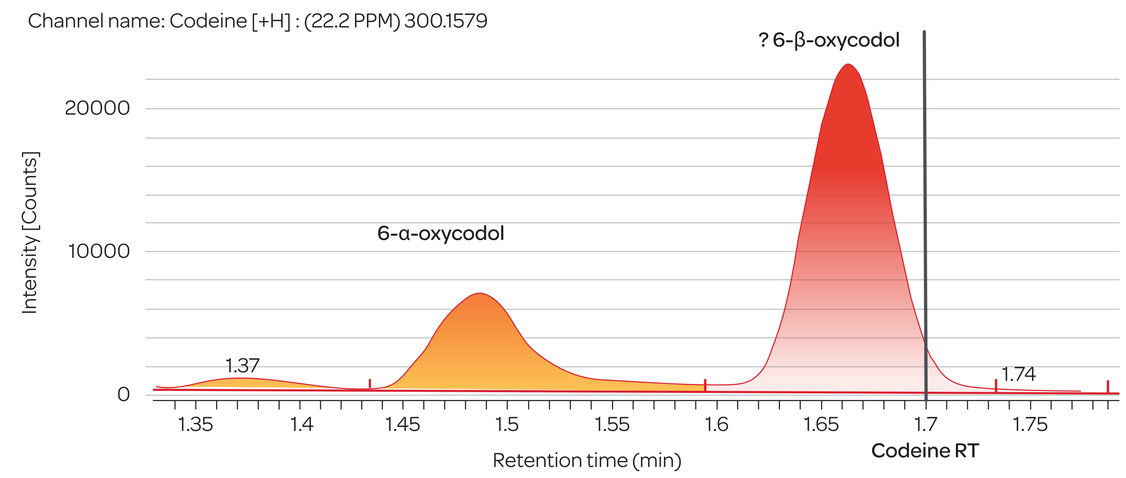

Figure 3. Identification of codeine interference peaks.

Conclusion

The potential for the false positive detection of codeine was identified in samples containing high concentrations of oxycodone and associated metabolites. In-source fragmentation (water loss) of 6-oxycodol was identified as a cause of false positive codeine interference. Resolution of this interference may involve modification of chromatographic separation or the use of unique product ion ratios. Acquisition of certified reference material is recommended for definitive confirmation of the interference.

References

1. Randall C. Baselt. Disposition of Toxic Drugs and Chemicals in Man, Twelfth Edition. Biomedical Publications, Seal Beach, CA

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