Introduction

When analyzing urine specimens for pain management compliance, it is important to identify any illicit substances that may put both the patient and physician at risk. The detection of an illicit substance in a patient undergoing a pain management regimen may result in dismissal from the program and discontinuation of prescribed medications. Identification of any and all compounds that may produce false-positive results is critical in distinguishing illicit substances from substances that may be found in over-the-counter medications and dietary supplements.

Two compounds that appeared to be Amphetamine and Methamphetamine were identified in two urine specimens collected from the same patient on separate occasions. Upon speaking with the physician, it was noted that the patient denied illicit use of these substances, but admitted to taking an over-the-counter dietary supplement known as "Supplement," which consisted of ingesting two pills orally. Urine was collected pre-ingestion and at one hour post-ingestion. The specimens were extracted per the validated Solid Phase Extraction (SPE) protocol. The urine specimens were quantitated using Analyst software and a Peak Review ion ratio report was generated.

Results and Discussion

"Supplement" Pill Dilution

To determine if these two compounds were being detected in these specimens, a bottle of "Supplement" was purchased from a local chain pharmacy. For quick determination, two aliquots of the pill mixture were placed in the test tube and diluted 10-fold in methanol. The mixture was vortexed, then centrifuged at 8000 g for 10 min, 10 μL aliquots of the mixture were then filtered and further diluted with 90 μL 0.1% Formic Acid in Water. 0.1% Formic Acid in Methanol (0.20 mL) in a 96-well plate.

Volunteer Urine Specimens

To verify that the compounds were present in patient urine after medication, two volunteers took a dose of "Supplement," which consisted of ingesting two pills orally. Urine was collected pre-ingestion and at one hour post-ingestion. The specimens were extracted per the validated Solid Phase Extraction (SPE) protocol. The urine specimens were quantitated using Analyst software and a Peak Review ion ratio report was generated.

Certified Standard Injections

BMPEA and Synephrine were both purchased from the vendor Phenomenex and were not identified as possible unique transitions. See Table 1 for transitions. BMPEA and Synephrine were spiked into patient/volunteer specimens, coupled with the absence of Synephrine’s fragmentations at 107.2 and 135.0 (see Figure 3). The fragmentations for BMPEA were identical to the spectra for Amphetamine, Methamphetamine, and to identify possible unique transitions. See Table 1 for transitions.

The results of the "Supplement" pill dilution revealed two peaks very close to the RTs of Amphetamine and Methamphetamine (Figure 2). This peak showed that 0.1 mg/mL of the mixture was diluted with DI water:methanol (80:20 v:v) in a 96-well plate.

Results and Discussion, cont’d

Conclusion

It was determined from these studies that the source of the false-positive Amphetamine was from the BMPEA in the over-the-counter dietary supplement "Supplement," due to the identical Retention Times observed in both the BMPEA standard and patient/volunteer specimens. The original hypothesis that the false-positive Methamphetamine peak was from Synephrine is yet to be determined and is an ongoing study. Differences in Retention Times between the Synephrine standard and patient/volunteer specimens, coupled with the absence of Synephrine’s two unique identifying transitions of m/z 150.1 and 150.0 in the volunteer’s specimens could possibly be due to the metabolism of Synephrine, or that some other ingredient not listed on the bottle with similar transitions was causing the false-positive. In conclusion, it was determined that "Supplement" does interfere with Amphetamine and Methamphetamine in the LC-MS/MS assay, and great caution is advised when releasing results to a physician, who may allow the patient to continue to receive treatment. Staff was trained on the identification of potential false-positives, with emphasis on the definitive criteria of Retention Time relative to the Amphetamine-D5 and Methamphetamine-D5 Internal Standards, as well as analytical ion ratios.

Table 1: Transitions monitored for Amphetamine, Methamphetamine, BMPEA, and Synephrine.

<table>
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<tr>
<th>Analyte</th>
<th>m/z</th>
<th>B</th>
<th>A</th>
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<tr>
<td>BMPEA</td>
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<td>138.1</td>
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<td>Methamphetamine</td>
<td>150.1</td>
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References