K28 Techniques for Screening and Confirmation of U-47700 and Flubromazepam in Two Non-Fatal Cases

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After attending this presentation, attendees will understand the need for extensive screening and confirmation capabilities in cases involving U-47700 and flubromazepam.

This presentation will impact the forensic science community by emphasizing the importance of using advanced instrumentation to detect emerging drugs of abuse when routine analyses are unremarkable.

A 21-year-old male (Case #1) was reported as acting erratic and had elevated vital signs when examined by a medical officer. He eventually confessed to using synthetic cannabinoids and nutritional supplements. The subject attributed his symptoms to an over-the-counter stimulant, but a search of his room found evidence of steroid use. A second 21-year-old male (Case #2) was reported as acting erratically and "shaking," but further investigative information was lacking. Specimens from both cases were subjected to volatile analysis by headspace/gas chromatography (HS/GC) equipped with a flame ionization detector (FID), immunoassay analysis (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, oxycodone, phencyclidine, 6-acetylmorphine, LSD), and a full scan GC/MS (GC/FS-MS) alkaline drug screen. Based on case history, further testing of specimens was performed by liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/qTOF). Presumptive positive samples were confirmed by GC/MS and LC/MS/MS. Testing results are listed in Table 1.

Table #1. Analytical Results	Case #1	Case #2
Volatiles (GC/FID)	None Detected	None Detected
Urine Immunoassay	+ Benzodiazepines	+ Benzodiazepines
Blood/Urine Benzodiazepine Confirmation (GC/MS)	None Detected	None Detected
Urine Basic Drug Screen (GC/FS-MS)	Chlorpheniramine Dextromethorphan	Diphenhydramine
LC/QTOF Drug Screen (Case #1: Blood) (Case #2: Urine)	U-47700 Flubromazepam Chlorpheniramine/Dextromethorphan	U-47700 Diphenhydramine
Urine Basic Drug Confirmation (LC/MS/MS)	63ng/mL U-47700 127ng/mL Flubromazepam 419ng/mL Chlorpheniramine 2330ng/mL Dextromethorphan	127ng/mL U-47700 5ng/mL Flubromazepam 130ng/mL Diphenhydramine
Blood Basic Drug Confirmation (LC/MS/MS)	36ng/mL U-47700 450ng/mL Flubromazepam 120ng/mL Dextromethorphan	20ng/mL U-47700 50ng/mL Flubromazepam
Blood Synthetic Cannabinoid Screen (LC/MS/MS)	None Detected	Not analyzed – insufficient history

These cases highlight the importance of using advanced instrumentation to compliment traditional analytical techniques. Based on in-house data, U-47700 has not shown cross-reactivity with immunoassay kits in fortified specimens below 10,000ng/mL. Flubromazepam will cross-react with benzodiazepine kits between 75ng/mL-100ng/mL. It is possible that flubromazepam metabolites are what caused the immunoassay benzodiazepine positive in Case #1. With a panel of mostly therapeutic analytes, the flubromazepam would be missed during the confirmation step. In these two cases, GC/FS-MS did not detect U-47700 or flubromazepam. Large, unidentified peaks were observed that were eventually attributed to U-47700 metabolites. Based on the case histories and initial testing results, additional testing was assigned to the LC/qTOF. By utilizing lower detection limits, expanded spectral libraries, and the benefits of LC over GC, U-47700 was detected in both cases, and flubromazepam was detected in Case #1. Both cases were then confirmed by LC/MS/MS, operating in multiple reaction monitoring (MRM) mode. Due to the increased sensitivity of the LC/MS/MS, urine from case #1 was analyzed even with limited volume left after all other testing had been completed.

These cases highlight difficulties that "designer" drugs can pose to traditional screening and confirmation techniques in the field of toxicology. As their popularity grows, laboratories need to utilize advanced instrumentation such as LC/qTOF and LC/MS/MS to detect these compounds. Comprehensive instrumentation and methodologies are vital in keeping up with emerging drug trends, sample volume limitations, and lower detection limits often required for these compound classes.

U-47700, Flubromazepam, LC/QTOF