

Validating an LC-MS/MS Method for the Inclusion of Bromazolam Screening in Routine Urine Drug Tests

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Background: With the emergence of bromazolam, a new benzodiazepine, in clinical cases within Island Health, there's a need to integrate it into routine urine drug screening. This study evaluates the feasibility of incorporating bromazolam into urine drug screening using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Targeted urine drug screening by LC-MS/MS utilized the AB SCIEX 4000 QTRAP mass spectrometer with electrospray ionization operating in positive mode. The method comprised a multiple reaction monitoring (MRM) survey scan, succeeded by an information-dependent acquisition-triggered enhanced product ion scan (EPI). The MRM survey scan served to identify potential drugs, while the EPI spectrum was subsequently compared to a drug library for compound confirmation. A 50 μ L urine sample was mixed with 50 μ L of methanol containing internal standards and 400 μ L of water. Subsequently, 20 μ L of the resulting dilution was injected for analysis following an 11-minute gradient elution HPLC run. Bromazolam or other drugs was considered positive if the combined score (retention time + library match) was equal to or greater than 70%. The method underwent evaluation for precision, cut-off limit, matrix effects, interference, and accuracy by spiking standards into drug-free urine. A total of 109 clinical specimens were tested during the method evaluation period.

Results: The study findings revealed a 6.2% intra-assay precision at a concentration of 10 ng/ml, demonstrating 100% agreement with spiked standards across 40 samples ranging from 10 to 10,000 ng/ml for bromazolam detection. No interference was observed from the other 74 drugs and their metabolites in the existing panel, and no carryover was detected even at concentrations up to 10,000 ng/ml. The mean matrix effect for bromazolam was determined to be -39%. Moreover, a cut-off level of 10 ng/ml for bromazolam in urine was established. During the method evaluation period, 37 out of 109 (34%) clinical specimens tested positive for bromazolam detection.

Conclusions: The integration of bromazolam into routine urine drug screening via LC-MS/MS not only broadens the scope of screening results but also ensures the reliability of detection. This advancement promises improved clinical service delivery and patient care.

Keywords: bromazolam; urine drug screening; LC-MS/MS method