Improved Screen and Confirmation Test of 7-Aminoflunitrazepam in Urine Specimens for Monitoring Flunitrazepam (Rohypnol) Exposure

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Abstract

Confirmed and alleged misuses of flunitrazepam (FM2, Rohypnol) have brought about serious interest in the development of an analytical methodology that can be effectively used for preliminary screen and confirmatory test of FM2 (or its metabolites) in urine specimens under high-volume settings. Reported methods do not serve this need well for the following reasons: (1) common benzodiazepine (BZ) immunoassays (IAs) have broad cross-reactivities toward widely prescribed BZs (and their metabolites) and are therefore likely to generate an unacceptable number of false positives and (2) because FM2 is typically used at low doses (1–4 mg), IAs with low cross-reactivities toward FM2 (and its metabolites) are likely to generate false-negative results. In this current study, a familiar and effective two-step IA/gas chromatography–mass spectrometry (GC–MS) approach is successfully developed and applied to clinical specimens. Cross-reacting characteristics of the following BZ IAs toward various BZs (and their metabolites) are evaluated focusing on their effectiveness in serving as the preliminary test reagent in a two-step testing protocol: TDx®, Beckman, CEDIA, Roche Cobas Integra, Emit® II Plus, and Cozart ELISA. Although other IAs show broad cross-reactivities toward various BZs and their metabolites, diazepam is the only non-FM2 derived compound that exhibits noticeable cross-reactivity toward Cozart ELISA reagent. Cross-reactivity data and data derived from studies conducted on a limited number of clinical specimens demonstrate that, when used to monitor FM2 exposure in a large population group (including those exposed to other BZs), Cozart ELISA has the potential of being as effective as (or better than) those currently used in various workplace drug-testing programs for monitoring respectively targeted drugs. Data derived from this study further suggest that 50 ng/mL apparent 7-aminoflunitrazepam (Cozart ELISA) and 30 ng/mL free 7-aminoflunitrazepam (GC–MS) are potentially effective preliminary test and confirmation test cut-offs. To maximize efficiency, it is further suggested that urine specimens are first diluted by a factor of 5 for the preliminary test in which a 10-ng/mL 7-aminoflunitrazepam standard is used as the assay's cut-off standard.

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Introduction

Recent popularity of flunitrazepam (FM2, Rohypnol) in rave parties (1), confirmed and alleged involvements in drug-assisted sexual assaults (2), and general interest in understanding its effects on human behavior and performance (3) bring about the need of a methodology that can be effectively used to analyze FM2 (or its metabolites) in urine specimens under high-volume settings.

Several immunoassays (IAs) (4,5) and gas chromatography–mass spectrometry (GC–MS) (6–9) protocols have been used to study the excretion profiles of FM2 following its ingestion under controlled conditions. Both approaches (IA and GC–MS) can provide valuable information under this circumstance—limited number of specimens containing no other benzodiazepines (BZs). However, these IAs are likely to generate unacceptable numbers of false-positive and false-negative results (see further discussion on these limitations in