Performance Characteristics of Two ELISAs for Preliminary Test of Urine Specimens from Patients under Flunitrazepam Treatment

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Learning Objective: Following the establishment of a two-step protocol [1] for high-volume analysis of urine specimens to detect flunitrazepam (FZ) exposure, this study compares the performance characteristics of two commercially available ELISA kits and ascertains corresponding cutoffs suitable for the immunoassay/GC-MS testing strategy.

Abstract: In an earlier study [1], we have demonstrated that Cozart Flunitrazepam Metabolite Micro-Plate EIA (Cozart Bioscience Ltd., Oxfordshire, UK), but not other general-purpose benzodiazepines EIA (such as TDx, Beckman, CEDIA, Cobas Integra, EMIT II Plus), can be effectively used for the preliminary test of urine specimens for FZ exposure. With FZ-specific ELISA from Immunalysis Corp (San Dimas, CA) now readily available, its performance characteristics are examined and compared to the Cozart product adapted in the earlier study. (Neogen Corp. (Lexington, KY) has also marketed FZ-specific ELISA. However, it was not included in this study because calibration standards needed for producing semi-quantitative data were not available.) A total of 144 urine specimens collected from 11 patients were studied to compare the performance characteristics of these assays. The resulting data were also evaluated to ascertain corresponding cutoffs suitable for the two-step immunoassay/GC-MS testing strategy. The concentrations of 7-amino-FZ in all specimens were first determined by GC/MS. These specimens were then diluted by a factor of 1, 5, 10, or 20 to bring the concentration of 7-amino-FZ in these specimens to the dynamic range of the immunoassays (50 ng/mL or less).

Shown in Figures 1A and 1B are correlation plots of the GC/MS data against the data derived from Cozart (A) and Immunalysis (B) reagents, respectively. The correlation of the two set of immunoassay data is further shown in Figure 1C. Resulting correlation parameters derived from Figures 1A and 1B are listed in Table 1.

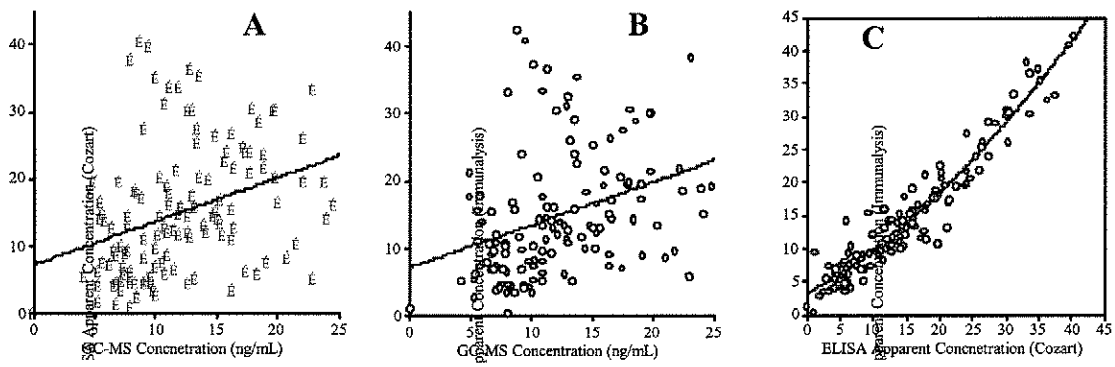

Figure 1. Correlation of GC-MS data against ELISA data derived from Cozart (A) and Immunalysis reagents and correlation of ELISA data derived from these two manufacturers.