D44 Performance Characteristics of Two ELISAs for Preliminary Test of Urine Specimens From Patients Under Flunitrazepam Treatment

Ya-Lei Chen, PhD, and Ray H Liu, PhD, Fooyin University, 151 Chin-Hsing Road, Tai-Liao, Kaohsiung, Taiwan 83101, ROC; Rea-Ming Yin, BS, Dong-Liang Lin, PhD, and Chung-Yi Wang, LLB, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan 83101, ROC; and Cheng-Hsing Chen, MD, Ping-An Hospital, Ping-An Hospital, Ping-Tong, Taiwan 83101, ROC*

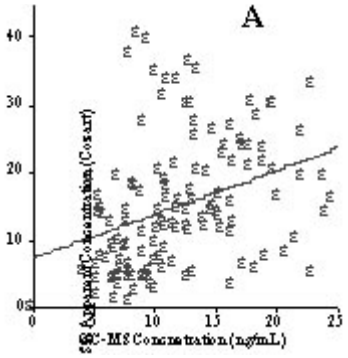
Following the establishment of a two-step protocol [1] for highvolume analysis of urine specimens to detect flunitrazepam (FZ) exposure, this study compares the performance characteristics of two commercially available ELISA kits and ascertains corresponding cutoffs suitable for the immunoassay/GC-MS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

This presentation will impact the forensic community and/or humanity by facilitating the development of an effective approach for high throughput detection of flunitrazepam exposure through the commonly adapted two-step immunoassay-GC/MS test strategy.

In an earlier study [1], the authors 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/GCMS 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.



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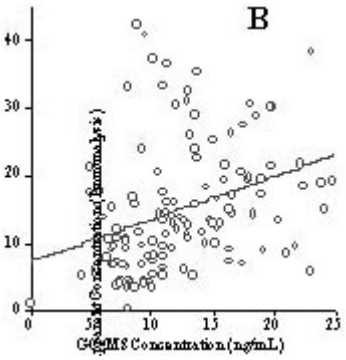

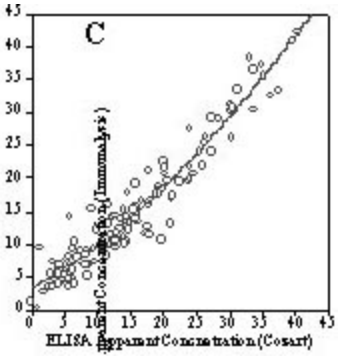

Table 1. Immunoassay-GC/MS data correlation parameters derived from data generated by two FZ-specific ELISA reagents

Manufacturer	Correlation equation	Correl. coef.	Immunoassay 7amino-FZ concn. (y) corresponding to 6 ng/mL GC/MS concn (x)
Cozart Immunoanalysis	y = 7.247 + 0.6465 x y = 8.294 + 0.5795 x	0.1176 0.09837	11.1 ng/mL 11.8 ng/mL

Data shown in Figure 1 and Table 1 suggest: (a) the performance characteristics of these two ELISA are very similar, with the Immunoassay product generating slightly higher responses (Figure 1C and last column of Table 1); (b) if all specimens are diluted by a factor of 5 before testing and 10 (or more precisely 11) ng/mL is adapted as the cutoff (corresponding to 50 ng/mL in the undiluted specimen) for the preliminary test, those tested positive are likely to contain 6 ng/mL (or 30 ng/mL in the undiluted specimen) 7-amino-FZ as determined by GC/MS. With both ELISA calibration optimized at the 0–25 ng/mL range, 5-fold specimen dilution and 10-ng/mL cutoff may work well for both products. The corresponding GC/MS may then be set at 30 ng/mL (undiluted specimen).

It was also noted that both ELISA are free from interference by many drugs (and their metabolitrs) that were prescribed to the patients in combination of FZ. These drugs include bromazepam (Lexotan), triazolam (Halcion), alprozolam (Xanax), clonazepam (Rivotril), venlafaxine HCl (Efexor), glibenclamide (Daonil), haloperidol (Haldol), zotepine (Lodopin), chlorpromazane (Wintermno), trihexyphenidyl (Artane), carbamazepine (Tegretol), lithium (Camcolit), and amlodipine basylate (Norvasc).

1. Wang P-H, Liu C, Tsay W-E, Li H-H, Liu RH, Wu T-G, Cheng W-J, Lin D-L, Huang T-Y, Chen C-H: Improved Screen and Confirmation Test of 7-aminoflunitrazepam in Urine Specimens for Monitoring Flunitrazepam (Rohypnol) Exposure; *J Anal Toxicol* 26:411-418; 2002.

Need Key Words

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