Urine Screening for Flunitrazepam: Applicability of Emit® Immunoassay

To the Editor:
Benzodiazepines are commonly abused drugs. Accordingly, urine specimens are often screened for benzodiazepines as part of drug-testing programs. However, immunoassays have different detection limits regarding the different benzodiazepines and their metabolites. Some benzodiazepines are therapeutically effective in low doses, also making detection difficult. Flunitrazepam belongs to this potent group of low-dosage benzodiazepines. The urine metabolites of flunitrazepam are reported to react weakly with most immunoassays [1]. Therefore, more time-consuming chromatographic analyses are often carried out to detect flunitrazepam intake. In this letter we report the applicability of the Emit® immunoassay (Behring Diagnostics, San Jose, CA) as a preliminary screening test for flunitrazepam. The described approach has proven to be both labor- and time-saving.

Flunitrazepam is prescribed primarily as a hypnotic, but is also used in some countries as a premedication in general anesthesia. Flunitrazepam is not an approved drug in the US. Recently, focus has been placed on flunitrazepam (designated as a Schedule I substance), or "Roofies," flunitrazepam (designated as a Schedule I substance), or "Roofies," and its popularity among misusers.

Positive 100 350 (200–1000) 180 (60–120) 80 (60–120)
Negative, but >0 22 130 (80–180) 370 370

Table 1. Detection of 7-aminoflunitrazepam in 122 authentic urine specimens.

Flunitrazepam is prescribed primarily as a hypnotic, but is also used in some countries as a premedication in general anesthesia. Flunitrazepam is not an approved drug in the US. Recently, focus has been placed on flunitrazepam (designated as a Schedule I substance), or “Roofies,” as a popular drug of abuse [2, 3]. In our country, as in many other European countries, the misuse of flunitrazepam has been known for many years. The drug is taken orally in tablet form, or the tablets may be dissolved in water for drinking or intravenous injection, often in combination with heroin. According to police reports, in our country the street price for flunitrazepam is ~30 times the pharmacy price, thus reflecting its popularity among misusers.

Table 1. Detection of 7-aminoflunitrazepam in 122 authentic urine specimens.

<table>
<thead>
<tr>
<th>Emit result</th>
<th>7-Aminoflunitrazepam</th>
<th>Median (range), µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative, but &gt;0</td>
<td>22</td>
<td>130 (80–180)</td>
</tr>
<tr>
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<td>100</td>
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The CV was 15% for a calibrator at the cutoff value and 13% for a control sample at ~270 µg/L. The analyses are run on a Monarch 2000 (Instrumentation Laboratory, Lexington, MA) at 30 °C in the semi-quantitative mode. Flunitrazepam is excreted as metabolites, primarily as 7-aminoflunitrazepam. The interaction of flunitrazepam metabolites with Emit immunoassay is reported as low [1] or not stated at all (Emit II Benzodiazepine Assay, instructions, 1994). Screening urine specimens with respect to flunitrazepam has thus been done in our laboratory by means of a highly sensitive gas-chromatographic (GC) method, while confirmation is performed with another analytical method (either HPLC or a different GC method). The cutoff was 60 µg/L (0.20 µmol/L) for 7-aminoflunitrazepam in the chromatographic analyses, with a CV of 11% at this concentration.

Annually we receive ~4000 urine specimens in which flunitrazepam analysis is requested. Emit screening was performed for other drugs of abuse, and in addition all these specimens were screened by GC. Only a few percent were positive. This raised the question of a simpler screening procedure, and we investigated the applicability of Emit benzodiazepine assay in this regard. A retrospective analysis showed that there had been a total of ~300 positive 7-aminoflunitrazepam specimens during the last 3 years. Approximately one-half of these samples contained another benzodiazepine drug as well and was thus unsuitable for evaluating the Emit results with respect to flunitrazepam metabolites. The specimens that were included in the study (122) apparently contained 7-aminoflunitrazepam alone. In addition, as these were authentic specimens, other flunitrazepam metabolites were present but were not analyzed for routinely. The Emit results of these samples are shown in Table 1. Most samples (>80%) containing 7-aminoflunitrazepam had Emit results above cutoff, and all samples had Emit results different from zero.

We now use Emit benzodiazepine immunoassay as a prescreening procedure with respect to flunitrazepam. A cutoff value at 60 µg/L has been chosen. If the Emit result of a specimen is below this value, we report a negative result, and the sample is not subjected to further analyses. With a higher Emit result, a GC screening is performed, and if positive is followed by a confirmation procedure. Also, an authentic urine control specimen containing ~60 µg/L 7-aminoflunitrazepam has been introduced. This (positive) control is analyzed together with all specimens, and the Emit result has to be higher than the negative calibrator. Otherwise, the prescreening condition is not fulfilled. Our data have shown that the risk of overlooking positive samples with an Emit cutoff at 60 µg/L is negligible. The use of Emit prescreening reduces the number of negative GC flunitrazepam analyses by as much as 80%. Some chromatographic screening analyses still must be done because of the simultaneous presence of other benzodiazepine drugs and metabolites with Emit responses. The percentage of positive flunitrazepam samples in the GC screening analyses has increased from 1.2% in 1993 to 20%
with the described approach. This increment cannot be explained solely by the reduced number of analyses due to prescreening, but also probably reflects increased misuse of these drugs, as well as more adequate and aimed practice regarding the request for drug analyses.

References

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Hydrogen Peroxide Interferes with Detection of Nitric Oxide by an Electrochemical Method

To the Editor:

Nitric oxide (NO) is a biologically active molecule synthesized from l-arginine in the presence of NO synthetase. Methods to quantify NO include those that use the Griess reaction, chemiluminescence, electron spin resonance, oxygen probing, and electrochemical detection using a porphyrin-based microsensor [1]. The latter method is advantageous for this labile molecule in real-time measurement and sensitivity [1, 2]. As the preparation of the microsensor is difficult, the use of this method has been limited to only a few institutions. Recently, an NO monitor with a resin microsensor based on the porphyrin-based model (Model NO-501; Inter Medical Co., Nagoya, Japan) became commercially available and has been used in several studies [3, 4].

In our studies of the effects of the redox state on NO generation in cultured cells, we found that hydrogen peroxide produced an electric current with the NO monitor. Fig. 1 illustrates the relationship between the current measured in response to different concentrations of hydrogen peroxide in phosphate-buffered saline at 25 °C. As shown, the current increases depending on the concentration of hydrogen peroxide and is higher than the current derived from the NO donor, NOC 5 (Wako Pure Chemical Co., Osaka, Japan).

This electrochemical method was originally reported to have a detection limit of 10 nmol/L, a linear response of up to 300 μmol/L, and a high selectivity for NO [1]. However, our results suggest the possibility of interference by hydrogen peroxide with the tested, commercially available, electrode. We speculate that this effect is caused by the reduced selectivity of the membrane of the commercially available resin microsensor, the composition of which is not published. This sensor is likely to be inappropriate for measurement of NO in biological material because hydrogen peroxide is so widespread in biological systems.

References

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Blood Lead Screening (Continued)

To the Editor:

I refute the allegations made by Verebey [1] that I have misquoted Wong et al. [2] and Schonfeld et al. [3], and summarize below the salient points.

I stated [4] that Wong et al. “found a positive bias of +3.4 μg/100 mL relative to inductively coupled plasma mass spectrometry . . . [and that they] attributed most of the bias to filter-paper blanks, which in 3 of 69 cases caused ‘apparent’ blood lead concentrations >40

Fig. 1. Relationship between the current measured and the concentrations of hydrogen peroxide in phosphate-buffered saline at 25 °C.

The current increases depending on the concentration of hydrogen peroxide. The relationship is described by the regression equation: current (pA) = −33.23 concentration of hydrogen peroxide (μmol/L) + 129.01. The current derived from hydrogen peroxide is higher than that from equimolar NOC 5 (Wako), an NO donor.