Comparison of the EMIT (Enzyme Multiplied Immunoassay Technique) Opiate Assay and a Gas-Chromatographic–Mass-Spectrometric Determination of Morphine and Codeine in Urine

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We examined 124 urine samples with the EMIT opiate assay kit and with a gas-chromatographic–mass-spectrometric determination (l) for morphine and codeine. With a cut-off value between positive and negative results at 0.5 mg (morphine equivalents) per liter for both methods, the EMIT assay gave 4.0% false positives and 5.6% false negatives when compared with 1. Lowering of the cut-off value for 1 to 0.1 mg/liter resulted in a decrease of false-positives to 1.6% and an increase of false-negatives to 6.4%, seemingly satisfactory for screening purposes.

Additional Keyphrases: double-beam spectrophotometers in EMIT technique • inter-method comparison • abused drugs • “kit” methods

Because of its high sensitivity and relative ease, the EMIT drug-abuse urine assay is widely used. However, the method has inherent disadvantages because of possible interferences of other drugs and urine constituents (e.g., enzyme inhibitors, salts, H+, or OH ions). These difficulties have been recognized and led to comparisons of the EMIT assay with other methods, such as radioimmunoassay (1–3), hemagglutination inhibition (2), fluorometry (2), and thin-layer chromatography (1–3).

All these methods also have their limitations with respect to specificity or sensitivity. For this reason it is desirable to compare results by the EMIT assay with those from a sensitive and specific method. We therefore decided to compare the EMIT assay for morphine with a gas-chromatographic–mass-spectrometric (GC-MS) determination, because this technique combines high sensitivity and specificity (4, 5).

Materials and Methods

The GC-MS combination was a model JMS-07 S instrument (JEOL Ltd., Tokyo, Japan) with multiple ion detection capabilities. The conditions were: 1 m x 3 mm (i.d.) glass column filled with 3% OV 17 on Chromosorb W-HP, 80–100 mesh; injection temperature, 260 °C; column oven temperature, 230 °C; temperature of connection to mass spectrometer, 200 °C; helium flow, 40 ml/min; electron impact energy, 30 eV.

As the recommended automatic instrumentation for the EMIT opiate assay was not available to us, measurements were made on a Shimadzu UV-200 double-beam recording spectrophotometer with thermostated cuvette holder (Shimadzu Seisakusha Ltd., Tokyo, Japan).

EMIT opiate kits were obtained from Syva Corp., Palo Alto, Calif. 94304.

Urines were obtained from outpatients attending a center for treatment of drug addicts (111 samples) and from inpatients of a general hospital (13 samples). The latter group of patients were receiving various medications, but no opiates.

EMIT Assay

Urine samples were, when necessary, centrifuged and the pH adjusted to 5.5–8.0.

The EMIT assay was slightly modified as follows. The bacterial suspension, prepared according to the EMIT procedure, was diluted by addition of 75 ml of EMIT buffer solution to 20 ml of suspension. Into a semi-micro cuvette (optical pathlength of 1.00 cm and 1.5 ml volume) were pipetted 0.95 ml of the diluted bacteria suspension, 0.10 ml of sample, and 0.05 ml of reagent A (antibody solution). After equilibration at 37 °C for 5 min, 50 μl of reagent B (enzyme solution) was added and the decrease in absorbance at 436 nm during the interval 10 to 50 s after this addition was measured from the recorder trace. The reference cell contained a similar cuvette filled with water.

The concentration of morphine equivalents was read from a calibration curve, prepared with EMIT standards in the same way. Urine samples giving a reading of more than 50 mg/liter were diluted with EMIT buffer and redetermined. On samples giving a reading of more than 0.5 mg/liter a blank lysisyme determination was performed, and if necessary the original reading was corrected accordingly. The within-run precision (CV) of the EMIT assay was 7% (n=38), the day-to-day precision 21% (n=29), determined in the range 0.5 to 50 mg/liter.

GC-MS Assay

The samples were hydrolyzed by adding to 15 ml of urine 1.5 ml of hydrochloric acid (8 mol/liter) and autoclaving for 30 min. The extraction and clean-up procedure were as described before (6). The dry residue was dissolved in 300 μl of methanol containing 3 mg of akekinetone (1-piperidino-1-phenyl-bicycloheptenyl-propanol-1) per milliliter as internal standard. Of this solution, 3 μl was injected into the GC-MS combination. The ions at m/e 294, 299, and 285 were monitored for akekinetone, codeine, and morphine, respectively. From the peak heights of these ions and calibration curves we calculated the concentration of codeine and morphine in the sample.

Akekinetone was chosen as internal standard because its retention time (.74) relative to morphine (1.00) and codeine (1.14) made it well suited for the production of a chromatogram containing three nicely discrete peaks, and because its mass spectrum contained an abundant fragment ion at m/e 294, well within range of the abundant molecular ions m/e 285 and m/e 299 from the spectrum of morphine and codeine, respectively.

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We first checked the specificity of the method by injecting 13 blank samples; we saw no increase in the monitored ions. Next, 15 positive samples were re-injected, and the m/z ions 244, 229, and 215 (for aminet, codeine, and morphine) were monitored. The concentration of morphine and codeine, calculated from the peak heights of these fragments, agreed with the results of the first determination within the limits that could be expected from the variance of the method. Because the EMIT opiate assay measures both morphine and codeine, but with different sensitivity, results of the GC-MS codeine determinations were converted into morphine equivalents by using the data supplied by Syva Corp. The within-run precision (CV) of the GC-MS assay was 5% (n = 25), the day-to-day precision 7% (n = 21).

Results and Discussion

Figure 1 summarizes our results. Notwithstanding the fact that the precision of each method is reasonable, the correlation between them is poor—not unexpectedly, since several factors influence the accuracy of the results, such as:

- conjugated morphine and codeine are determined completely after hydrolysis by the GC-MS method; the EMIT method is less sensitive for these conjugated forms than for the free substances;
- the EMIT method has no absolute specificity, so cross-reactions with other substances present in urine may be possible;
- the antigen–antibody coupling or the lysozyme activity may be influenced by substances present in urine;
- preparation of samples for the GC-MS determination causes a loss of morphine and codeine; for morphine this loss is 6–15% (15 recovery determinations), for codeine 4–12% (15 recovery determinations); and
- dilution of urine samples when EMIT readings exceed 50 mg/liter may introduce some error (e.g., by changing the electrolyte content or the concentration of other substances in the sample).

For practical purposes only the results in terms of positive-negative are of interest. If a cut-off level of 0.5 mg/liter, as recommended for EMIT, is selected for both methods, and the results of the GC-MS method are accepted as true, area A of Figure 1 contains the falsely positive EMIT readings and area D the falsely negative. Expressed as percentage of the total number of determinations this amounts to 4.0% false-positives and 5.6% false-negatives.

It is not practical to select a much lower cut-off value for EMIT, because the difference in absorbance between negatives and low positives then becomes very small. For the GC-MS method it is possible, and also desirable, to select a lower value, because the presence of even a very small amount of morphine gives a positive result. With an arbitrarily chosen cut-off level of 0.1 mg/liter the falsely positive results decrease to 1.0%, the falsely negative increase to 6.4%.

Because in many practical situations a falsely positive result has more consequences than a falsely negative, and especially makes confirmation by another method necessary, one will generally try to limit the number of false-positives, even at the cost of an increased number of false-negatives. Thus, one may conclude from the results of the examined series that the EMIT method can be useful for the surveillance of drug abuse.

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References