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Validity Testing of Commercial Urine Cocaine Metabolite Assays: IV. Evaluation of the EMIT® d.a.u.™ Cocaine Metabolite Assay in a Quantitative Mode for Detection of Cocaine Metabolite

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ABSTRACT: The EMIT® d.a.u.™ cocaine metabolite assay (EMIT dau) was evaluated in a quantitative mode for analysis of clinical specimens obtained after controlled cocaine administration to human subjects. The quantitative results showed high concordance with those of gas chromatography/mass spectrometry (GC/MS) assays of the same specimens for benzoylecgonine, and no false positive or false negative results were obtained. The evaluation also included analysis of standardized solutions containing benzoylecgonine, cocaine, and other cocaine metabolites and isomers. The EMIT dau antibody demonstrated high selectivity for benzoylecgonine. The precision was somewhat less than that reported earlier for other commercial cocaine metabolite immunoassays. Quantitation of initial screening results from EMIT dau testing can serve as a useful guide for confirmation by GC/MS in forensic science urine testing.

KEYWORDS: toxicology, cocaine, EMIT®

The enzyme-multiplied immunoassay technique (EMIT®) is a procedure for analysis of drugs in biological fluids. The procedure is based on competition between an antigen (drug) and an antigen labeled with an enzyme for limited binding sites on an antibody. The activity of the enzyme is diminished when bound to the antibody; thus, the drug concentration can be measured in terms of enzyme activity. The selectivity of the antibody for the target analyte and related substances is one of the major factors determining the accuracy of quantitative results [*1*]. Because of antibody cross-reactivity with other analytes, many immunoassays have been found to overestimate specific analyte concentrations; however, underestimation also can occur [*2*]. As a result of the inherent lack of true specificity in many immunoassays, confirmation by a second method specific for the analyte of interest is considered necessary in forensic science testing. The present method of choice for confirmation is gas chromatography/mass spectrometry (GC/MS) [*3*].

Although EMIT has been used as a quantitative assay [*4*], it is more commonly used in the qualitative mode as the initial test in screening for drugs of abuse (EMIT® d.a.u.™), as outlined in the Mandatory Guidelines for Federal Workplace Testing [*5*]. Quantitation

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of initial urine screening results may be useful as a guide when performing confirmation by GC/MS, since accurate quantitation across a broad range of concentrations is often necessary. Highly concentrated specimens may be diluted to concentrations appropriate for standard curve analysis, thus obviating a second GC/MS analysis.

In recent studies, the authors of this paper and others evaluated the EMIT® d.a.u.™ cocaine metabolite assay (EMIT dau) in the qualitative mode for detection of cocaine metabolite in urine [6,7]. Since quantitation can be useful, even when the test is employed as the initial screen, reanalysis of the EMIT dau data in a quantitative fashion was needed. The present report documents the performance of EMIT dau when evaluated in the quantitative mode.

Materials and Methods

Specimens and Assay

Clinical specimens obtained from five human subjects after intravenous administration of cocaine, together with control urines containing known amounts of cocaine metabolites and derivatives, were analyzed under blind conditions in randomized order for the presence of cocaine metabolite [5]. A total of 148 clinical specimens and 142 standardized urines (spiked) comprised the set. The assay was performed on a Syva AutoCarousel™ equipped with a Syva spectrophotometer (Model S-III) and a Syva laboratory processor (Model 6500) with EMIT dau reagents (Syva Co., Palo Alto, CA). At the start of each day, duplicate negative, low, and medium calibrators were assayed under the criterion that each duplicate assay could not differ by more than 6 absorbance units. Thereafter, duplicate low calibrators were assayed with each batch of 60 specimens. If the repeat low calibrator differed by more than 6 absorbance units, the instrument was recalibrated. All specimens were initially analyzed as positive or negative relative to the low calibrator response (300 ng/mL cutoff). Quantitation of the EMIT dau response was performed by fitting calibrator responses with a four-parameter log-logit fitting routine. The specimen responses were used to calculate estimates of benzoylecgonine equivalents from the resulting standard curve.

Results and Discussion

The accuracy of the EMIT dau assay for measurement of cocaine metabolite (benzoylecgonine) was assessed across a wide concentration range (0 to 5000 ng/mL). Each concentration was assayed in triplicate and the means are reported in Table 1. The precision at each concentration is indicated by the percent coefficient of variation (%CV). Quantitative data from the GC/MS assays of the same specimens also are included for comparison. All data points are shown in the standard curve plots of Fig. 1 for both EMIT dau and GC/MS, along with least-squares linear regression data. Although the detection limit of the EMIT dau assay for benzoylecgonine was estimated to be approximately 30 ng/mL (determined on the Syva ETS™ System, Syva Co.), specimens containing 50 ng/mL were not detected in the present study with the AutoCarousel™. It is likely that differences in performance characteristics between these systems account for this discrepancy. At the 300-ng/mL cutoff and above, the mean quantitative values obtained by EMIT dau (Table 1) were elevated with respect to the target concentrations and with respect to GC/MS assay data. This is somewhat surprising since the slope of the regression line of the EMIT dau standard curve (Fig. 1) is very close to unity (1.08 versus 1.00), indicating a lack of relative bias. The overall precision of the EMIT dau assay was generally less than that of the GC/MS assay and also was less than that observed for other commercial immunoassays reported earlier [7].

TABLE 1—*Accuracy of EMIT dau assay determinations of benzoylecgonine concentrations in standardized control urines versus GC/MS assay determinations.*

Benzoylecgonine Concentration, ng/mL	Mean Assayed Concentration, ng/mL[a]			
	EMIT dau	%CV	GC/MS[b]	%CV
0	0	0	0	0
50	0	0	73.7	33.4
150	121.5	12.1	119.3	24.3
300	344.2	15.4	328.3	5.9
500	858.2	29.0	525.7	8.0
750	1017.5	19.9	747.3	8.0
1000	1262.0	30.3	1045.3	3.5
2500	2648.8	16.1	2569.3	4.0
5000	>3300.0	...	5904.7	19.9

[a]$N = 3$.
[b]Reported earlier by Cone and Mitchell [7].

Quantitative assessment of the cross-reactivity of the EMIT dau antibody with benzoylecgonine, cocaine, metabolites, and isomers revealed that the antibody is highly selective for benzoylecgonine. The estimated cross-reactivity for benzoylecgonine when tested at 300 ng/mL was 114.7%. Cocaine, isomers of cocaine, and other metabolites of cocaine had <1% cross-reactivity when tested at 5000 ng/mL. The compounds included in testing were the following: *l*-benzoylecgonine; *l*-cocaine; *l*-ecgonine methyl ester;

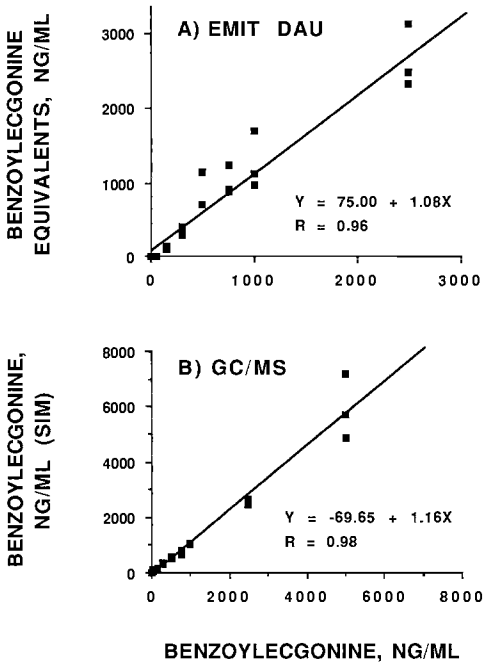

FIG. 1—*Benzoylecgonine standard curves obtained* (a) *by EMIT dau and* (b) *by GC/MS. Benzoylecgonine standard solutions were prepared in control urine in triplicate and analyzed together with clinical specimens in randomized order under blind conditions.*

l-ecgonine; *l*-benzoylnorecgonine; *l*-norcocaine; *d*-cocaine; *d*-pseudococaine; *l*-pseudococaine; *l*-pseudoecgonine methyl ester; and *d*-pseudoecgonine methyl ester.

The concordance of the quantitative EMIT dau data for cocaine metabolite in clinical urines with data from the specific GC/MS assay for benzoylecgonine is shown in Table 2. There was a high concordance of EMIT dau data with the GC/MS assay data. These data essentially are a repetition of the qualitative data reported earlier [6]. There were no false positives (specimens testing positive which had ≤20 ng/mL of benzoylecgonine by GC/MS assay) and no false negatives (specimens testing negative which had ≥300 ng/mL of benzoylecgonine by GC/MS). A combined plot of all clinical specimens quantitated by EMIT dau (<3300 ng/mL benzoylecgonine equivalents, $N = 127$) versus GC/MS assay results for benzoylecgonine is shown in Fig. 2. The solid and dotted lines superimposed represent least-squares regression fits of EMIT dau assay results for the clinical specimens and benzoylecgonine urine standards, respectively. These data also attest to the apparent lack of bias in the EMIT dau assay for benzoylecgonine.

Individual subject excretion plots of cocaine metabolite by EMIT dau and GC/MS assay for benzoylecgonine are shown in Fig. 3. It should be noted that Subject A (Panel A) received a second cocaine administration 24 h after the 20-mg dose and, hence, tested positive throughout the testing period. EMIT dau estimates of cocaine metabolite declined rapidly and were negative (300 ng/mL cutoff) within 48 h. The individual detection times (in hours) from administration of the drug to the last positive specimen were the following: Subject B, 31.2; Subject C, 36.6; Subject D, 39.1; and Subject E, 36.3. The mean ± standard error of these data, 35.8 ± 1.7 h, compares favorably with

TABLE 2—*Concordance of quantitative results of the EMIT dau assay of 148 clinical specimens for cocaine metabolite with GC/MS assay results for benzoylecgonine.*

EMIT dau Result, ng/mL	GC/MS Concentration, ng/mL					
	>300	299–200	199–150	149–100	99–20	<20
≥300	61	6	0	0	0	0
<300	0	2	2	3	35	39

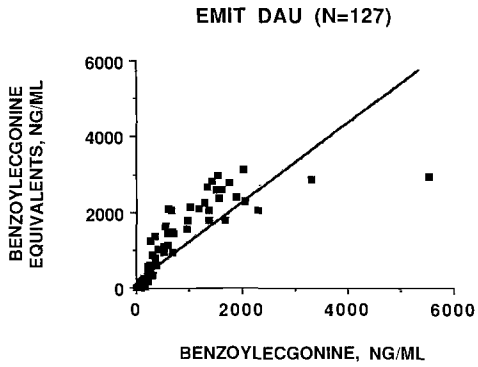

FIG. 2—*Methods comparison for quantitative data from the EMIT dau assay versus the GC/MS assay for benzoylecgonine. The solid line shows the least-squares regression fit of data points. The dotted line shows the least-squares linear regression fit for the benzoylecgonine standard curve from Fig. 1a.*

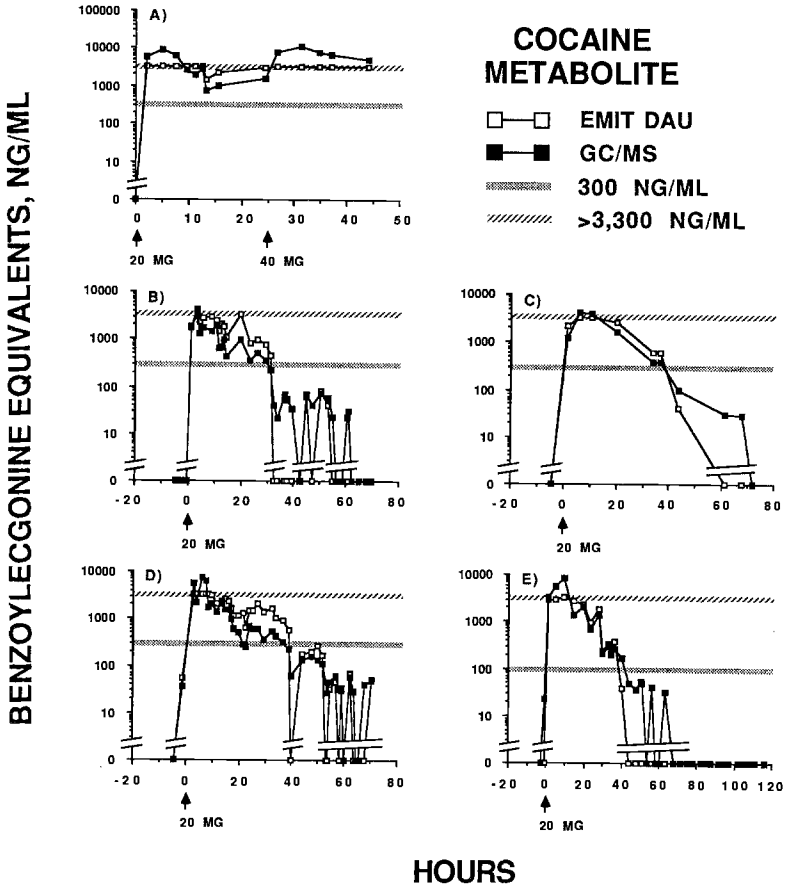

FIG. 3—*Quantitative data from the EMIT dau assay and GC/MS assay for benzoylecgonine in urine of Subjects A through E after intravenous cocaine administration. All the specimens were analyzed in randomized order under blind conditions. Specimens with benzoylecgonine equivalents equal to or higher than 3300 ng/mL by EMIT dau are reported as >3300 ng/mL.*

the corresponding mean GC/MS detection time of 32.6 ± 2.3 h. These data are identical to those reported earlier for the qualitative assessment of the EMIT dau assay [*6*].

Overall, the EMIT dau assay for cocaine metabolite performed well in the quantitative mode for estimation of benzoylecgonine concentration. Although the assay tended to overestimate the concentration in spiked specimens and, to some degree, in clinical specimens, the concordance of quantitative results with GC/MS assay results for benzoylecgonine was high, probably as a result of the high selectivity of the antibody. However, the precision was less than that observed for other commercial cocaine metabolite assays evaluated in the quantitative mode [*6*]. Quantitative analysis of EMIT dau data for cocaine metabolite would appear to serve as a useful guide for confirmatory analysis.

Acknowledgments

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