Evaluation of Immunoassays for Semiquantitative Detection of Cocaine and Metabolites or Heroin and Metabolites in Extracts of Sweat Patches

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Abstract

Two types of immunoassays, radioimmunoassay (RIA) and microplate enzyme immunoassay (EIA), were compared for their ability to detect and quantitate cocaine and metabolites or heroin and metabolites in extracts of sweat patches. Experiments used sweat patches that had been fortified with cocaine, benzoylecgonine (BE), and e cogine methyl ester (EME) or 6-acetylmorphine (6-AM), heroin, and morphine. Assays were first evaluated for sensitivity in detection of the analyte(s) known to be excreted in sweat (cocaine >> BE and EME; 6-AM > heroin > morphine). The cocaine metabolite RIA had cross-reactivity for cocaine > BE > EME, and the cocaine metabolite EIA had cross-reactivity for BE > cocaine > EME. The RIA, having greater sensitivity for COC, was studied further. Optimal linearity was 4 to 200 ng/patch, and quantitation within these limits at 4, 75, and 150 ng/patch had intrarun %CVs within 7.8% and percent targets within 15% and inter-run %CVs within 13.5% and % targets within 13%. The opiate RIA had cross-reactivities for morphine >> 6-AM and heroin. The opiate EIA had cross-reactivities for 6-AM and heroin of 42 and 28% relative to morphine, respectively. The EIA, having greater sensitivity for 6-AM and heroin, was studied further. The limits of detection ranged from 1.7 to 24.7 ng/patch, and the lower limits of quantitation ranged from 7.3 ng/patch to beyond the linear range. The assay, however, had consistently good precision at 4 and 5 ng/patch, and optimal linearity was established from 4 to 100 ng/patch. With controls at 5, 25, and 90 ng/patch, both intrarun and inter-run precision were acceptable. Quantitation was accurate at 5 and 25 ng/patch, but the 90 ng/patch controls were consistently < 70% of target. Because our studies focused on the assays that had greater sensitivity for the analytes excreted in sweat, we did not fully evaluate the cocaine metabolite EIA or the RIA opiate screen and therefore cannot make any comment on the usefulness of these assays for detecting analytes in extracts of sweat patches beyond predicting that they will have less sensitivity. Both the cocaine metabolite RIA and opiate EIA had the ability to detect analytes known to be extracted from sweat patches.

Introduction

Sweat is a significant route of elimination of some, particularly basic, drugs (1). The collection of sweat for analysis has been a limiting factor in these studies. With the use of occlusive patches containing absorbent material, drugs of abuse such as amphetamines (2,3), methadone (4), phenobarbital (5), phencyclidine (6), alcohol (7), and cocaine (8) were detected in sweat. As these patches did not allow the passage of water, they had disadvantages that included a change in the matrix environment and irritation for the wearer (9) that limited the duration of patch application.

The development of a nonocclusive patch through which water and smaller volatile molecules could pass allowed for prolonged attachment of the patch to subjects. This type of patch has been used for a number of studies on the detection of cocaine and metabolites (10-12), opiates (10,13,14), amphetamines (15,16), phenobarbital (13), benzodiazepines (17), and general drug use in opiate users being treated with buprenorphine (18). The sweat patch has been advocated for circumstances where monitoring cumulative drug use over a period of a week(s) would be useful. These include substance abuse treatment programs and the criminal justice system programs.

The use of sweat patch findings may be limited, as has been found for drug findings in urine. Interpretations of concentrations of drugs in sweat will likely have limited value in determining the dose or frequency of use, much as is the case with urine samples (19,20). In substance abuse treatment programs, however, the use of quantitative measurements to monitor trends of increases and decreases in drug use over time has been proposed as a better measure of the efficacy of treatment.

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(21,22), suggesting that there may also be some utility in (semi)quantitative measurements in sweat. This requires assays with relatively good selectivity for the analyte in question, as can be achieved with mass spectrometric methods of analysis for most drugs or drug metabolites. This is also the case for immunoassays designed for the detection of cocaine metabolite in urine. Indeed, the accuracy and precision of immunoassays for semiquantitative detection of benzoylecgonine (BE) have been demonstrated (23,24).

A similar application of semiquantitative immunoassays to sweat patches would require consideration of the major analyte(s) excreted in sweat. After cocaine use, the parent drug cocaine has been detected in greater quantities than the metabolites BE and ecgonine methyl ester (EME) (10). Following heroin use, the pattern of excretion is not as clear. During the first 24 h, heroin is consistently found at higher concentrations than 6-acetylmorphine (6-AM) or morphine (10,14). However, when the patch is worn for longer times, 6-AM concentrations will often exceed those of heroin. Of 27 opiate-positive subjects from 2 studies in which patches were worn for at least 3 days, 6-AM was highest in 13 subjects, heroin was highest in 6 subjects, and morphine was highest in 8 subjects (10,18). When 6-AM was present at the highest concentration it was often there at concentrations that exceeded the other analytes by 2–10-fold. It is also important to note that there were cases where either morphine, 6-AM, or heroin was not detected in extracts of these patches.

It therefore seemed that an immunoassay with good sensitivity and specificity for cocaine would be preferable for detection of cocaine use, and an immunoassay that had sensitivity for heroin, 6-AM, and morphine would be useful for detection of heroin use. Assays that had lower limits of quantitation (LLOQ) that would approximate the reported 1 to 5 ng/mL (0.4 to 2 ng/patch) LLOQs of gas chromatographic–mass spectrometric (GC–MS) methods (10,12,18,25) would also be useful. To date, evaluations of immunoassays for detection of drugs in sweat patch extracts have been limited to the qualitative detection of the respective drugs in STC Technologies, Inc. (Bethlehem, PA) cocaine metabolite and opiate microplate enzyme immunoassays (EIA) (12,26). Although Burns and Baselt (11) demonstrated the utility of the Diagnostic Products Corp. (DPC, Los Angeles, CA) cocaine metabolite radioimmunoassay (RIA) to detect cocaine in sweat patch extracts, the study did not include an evaluation of the sensitivity, precision, and accuracy of the RIA. We evaluated the RIA of DPC (27,28) and the microplate EIA of STC (29,30) for their ability, with or without modifications, to detect the predominantly excreted analyte(s). The assays that showed the better sensitivity were further evaluated for their ability to accurately and precisely quantitate the predominant analyte. This is an initial step in addressing the question of whether immunoassays can accurately and precisely quantitate an analyte extracted from sweat patches. The precision and accuracy studies were therefore limited to the assessment of patches fortified with the predominant analytes, cocaine and 6-AM. Additional studies with the selected assays will be needed to determine their utility for quantitative analysis with samples collected from subjects.

**Methods**

**Materials**

Cocaine, BE, EME, heroin, 6-AM, and morphine were purchased from Radian (Austin, TX) as free-base solutions. Cocaine hydrochloride was also purchased from Sigma Chemical Co. (St. Louis, MO). 6-AM hydrochloride was also purchased from Altech (Deerfield, IL). Pharm Chek® drugs-of-abuse patches were purchased from Pharm Chem Laboratories (Palo Alto, CA). Polypropylene vials (5 mL) for extractions were purchased from VWR Scientific Products (Salt Lake City, UT). The Cocaine Metabolite and Opiates Screen Coat-A-Count RIAs were purchased from DPC. The cocaine metabolite and opiate microplate EIAs were purchased from STC.

**Preparation of patches**

Stock solutions of 100 µg/mL of cocaine, 100 µg/mL BE, and 100 µg/mL EME in methanol were used to prepare working solutions that contained either 0.1 µg/mL or 1.0 µg/mL each of cocaine, BE, and EME or cocaine only in Milli-Q water. Stock solutions in methanol of 100 µg/mL of heroin, 100 µg/mL 6-AM, and 100 µg/mL morphine were used to prepare working solutions that contained either 0.1 µg/mL or 1.0 µg/mL each of heroin, 6-AM, and morphine or 6-AM only in Milli-Q water. For control patches, stock solutions at 1.0 mg/mL cocaine in methanol and 0.1 mg/mL 6-AM in methanol were prepared using reference material purchased from a different vendor than that used to prepare calibrators. These stocks were then used to prepare working solutions at 1.0 and 0.1 µg/mL in Milli-Q water. The concentration of analytes in working solutions was confirmed by GC–MS.

After removal from the packaging, the patches were labeled and placed adhesive-side up on clean territowels. The appropriate amount of working solution was applied to the absorbent pad using calibrated Pipetmans and the patches were allowed to dry for at least 1.5 h. The absorbent pad was then removed from the adhesive layer with forceps and rolled up with a wooden application stick. The rolled pad was then placed into the 5-mL polypropylene vial, and the vial was capped and stored at −20°C until analysis.

**Extraction of patches**

Patches were extracted as described by the manufacturer. Extraction buffer (2.5 mL, methanol/0.2M pH 5 sodium acetate, 75:25, v/v) was added to each extraction vial. The tubes were recapped and placed on an orbital shaker for 30 min. Extract was taken directly from these vials for further analysis.

**RIA**

The RIAs were initially performed as described by the manufacturer (27,28), where 25 µL of sample was first added to antibody labeled Coat-A-Count tubes, followed by 1.0 mL of 1125-tracer. The tubes were then allowed to incubate for 2 h (coca- ine) or 1 h (opiates). The contents were decanted, and then the tubes were counted on a Cobra gamma counter (Packard). As shown in the Results and Discussion section, we subsequently found that the assay of sweat patch extracts for cocaine could be improved by adding the tracer first, then sample, and increasing the sample volume to 100 µL.
EIA

The EIAs were performed using the manufacturer's instructions (29,30) as follows. Seventy-five microliters of pre-buffer was added to each well, followed by 50 μL of sample and 100 μL of enzyme (horseradish peroxidase) conjugate. The plates were then incubated for 30 min at room temperature and the contents dumped. The wells were then washed 6 times with 300 μL water per well using a Bio-Tek EL-404 microplate washer. The plate was then blotted, and 100 μL of substrate, 3,3',5,5'-tetramethylbenzidine, was added to each well, after which the plate was incubated in the dark for 30 min. The reaction was then stopped with 100 μL of 1M sulfuric acid per well, and the absorbances at 450 nm and 630 nm reference were read using an Ebx 808 plate reader equipped with a Bio-Tek KC4 data-handling program.

Calculations

The data for all assays have been expressed as B/Bo, using extracts of blanks for the Bo, whether the original data were expressed as absorbance or counts per minute. When concentrations were calculated both plots of B/Bo versus log concentration and logistic curves were applied as indicated in the test. The calibrators prepared in house were used for calibration curves, with manufacturer's calibrators run concurrently in most assays for comparative purposes.

Statistics

Statistics were performed using a one-way analysis of variance (ANOVA) at \( p < 0.05 \) and the Tukey post-hoc test also at \( p < 0.05 \).

Results and Discussion

Reports on sweat patches have used units of nanograms per patch or nanograms per milliliter of extract. We have chosen to use nanograms per patch because it refers to the primary sample and will give results that can be compared to other studies even if the extraction procedure uses a different volume. With most of the recent studies using a 2.5-mL extraction, the results in nanograms per patch are equivalent to the results in nanograms per milliliter of extract multiplied by the extract volume of 2.5.

The studies on the detection of cocaine and metabolites are presented first, followed by the studies on detection of heroin and metabolites.

Detection of cocaine, BE, and EME under normal conditions

After ingestion of cocaine, the three main analytes detected in sweat are the parent drug, cocaine, and the metabolites BE and EME (10). The RIA and EIA were first assessed, using the procedures of the manufacturer, for their ability to detect cocaine, BE, or EME at concentrations up to 100 ng/patch (Figure 1). With the RIA, cocaine produced a significant signal that was evident at the lowest concentration, 2.5 ng/patch, whereas BE was not detected until approximately 50 ng/patch and EME was not detected at 100 ng/patch (Figure 1A). With the EIA, BE gave the strongest signal, but was closely followed by cocaine. EME started showing a concentration-dependent signal at approximately 50 ng/patch (Figure 1B). With the EIA, BE gave the strongest signal, but was closely followed by cocaine. EME started showing a concentration-dependent signal at approximately 50 ng/patch (Figure 1B).

Though this experiment was not purposely designed to determine cross-reactivities in the usual manner (i.e., BE did not have a sufficient linear range to determine cross-reactivities across a number of concentrations), they could be approximated by comparison of concentrations of cocaine and EME that gave rise to similar signals of BE. This provides a basis for comparison of our results with those previously reported. With the RIA, an average B/Bo for the BE 100 ng/patch of 0.70 was comparable to the 0.74 B/Bo for cocaine at 5 ng/patch (approximately 2000% cross-reactivity of cocaine relative to BE). EME was not tested at a
high enough concentration to detect any cross-reactivity, suggesting a cross-reactivity relative to BE of less than 1% at 100 ng/patch. These findings are consistent with those reported by the manufacturer (27). The EIA showed cross-reactivity for cocaine that was only slightly less than the 102% reported by Spiehler et al. (12). The EME signal at 100 ng/patch was between that of BE at 5 and 10 ng/patch. This too is consistent with the reported cross-reactivity for EME of 15% (12).

Attempts to enhance cocaine metabolite EIA sensitivity

When testing matrices for which an immunoassay has not been intentionally developed, we found that modifications to the manufacturer's recommended procedures may enhance the sensitivity (31,32). Because our initial experiments suggested that the RIA was going to be more sensitive to analytes in sweat patch extracts, we wanted to see if we could enhance the sensitivity of the EIA with changes in conjugate volume, sample volume, or incubation time. The size of the microplate well precluded experiments on increasing sample or reagent volume. Decreases in conjugate volume from 100 to 50 μL had minimal effect on the detection of cocaine, but increased the B/Bo for BE at 20 ng/patch and EME at 20 and 50 ng/patch (Table I). When comparing 50 to 75 μL of sample volume, no effect was found except for a slightly increased B/Bo for EME at 50 ng/patch (Table I).

The development of color in the EIA was time dependent, reaching a maximum at approximately 45 min (Figure 2A). From 20 min on, however, the B/Bo for cocaine, BE, and EME were not changed (Figure 2B). These results suggested that modifications that could be readily employed in the lab were not going to enhance the sensitivity of the EIA for analytes encountered in sweat patch extracts. Although these findings indicated that the RIA would be more sensitive towards detection of the main analyte, cocaine, excreted in sweat, they do not rule out the value of the EIA for screening purposes. These have been well established (12). Our aim, however, was to first find the more sensitive assay and evaluate it for potential as a quantitative assay, we therefore concentrated further efforts on the RIA methodology. Subsequent studies with the RIA, using samples collected from cocaine-using subjects, in which both the qualitative and quantitative nature of the RIA are evaluated could then be compared to the qualitative evaluation of the EIA for use with sweat patches (12).

Effect of sample volume on the cocaine metabolite RIA

We explored the effect of sample volume on the RIA detection of cocaine, BE and EME (Figure 3). When comparing the addition of 25 μL, as recommended, to 100 μL at concentrations from 5 to 20 ng/patch, a dramatic drop in B/Bo was found for all three analytes.

Figure 2. The effect of incubation time on the absorbance (A) and B/Bo (B) for the detection of cocaine (○), BE (□) and EME (△) with the EIA. Values are the mean ± SD for three patches per concentration. * - Statistically different from other time points for that analyte.

Figure 3. Sample volume effect on RIA detection of cocaine (○), BE (□), and EME (△). A Comparison of 25-μL (open symbols) and 100-μL sample volumes (closed symbols) at different concentrations. Values are mean of two patches. B, Comparison of different sample volumes from 20 ng/patch. Values are the mean ± SD of three patches at each volume. * - Statistically different from other volumes in experiment B.

Figure 4. The effect of modification of aliquoting procedure on variation of sweat patch extract RIA results. In experiments 1 and 2, all 80 aliquots (20 per concentration) were put into the tubes before tracer was added; RIA lot #1 (○) and RIA lot #2 (□). In experiment 3 (△), 20 samples were aliquoted (5 per concentration) and then tracer added; this was repeated until all 80 samples had been aliquoted. In experiment 4 (△), tracer was first added to all tubes and then the 80 aliquots (20 per concentration).
establishing the limit of detection (LOD) and the lower limit of quantitation (LLOQ) for the cocaine metabolite RIA

To establish the LOD and LLOQ for the RIA, 20 patches per concentration were fortified at 0, 2, 4, and 5 ng cocaine/patch. The patches were extracted, and 100-μL aliquots of the extract were tested by RIA. The variance of the blanks would then be used to establish the LOD and LLOQ at 3 and 10 SDblank, respectively. The converse difference would also be determined. This was done to ensure that a concentration suitable for the LLOQ would have variance that was at least 10 SDLLOQ from the blank. In our first experiment, we found considerable variation at all concentrations (Figure 4). Using the 3 SDblank (0.318 B/Bo) would have resulted in an LOD at the highest end of the dynamic range. We repeated the experiment using a different lot of the RIA and found similar results (Figure 4). The possibility that the length of time that the extract was sitting in the tube may have caused this variation was explored by aliquotting 20 patch extracts (5 per concentration) at a time, adding tracer, and then repeating until all 80 extracts had been aliquotted. This reduced the variance of the blanks (Figure 4) such that an LOD at 3 SDblank could be set at a B/Bo of 0.583, but an LLOQ at 10 SD would still be outside the possible dynamic range. Furthermore, we were still not obtaining a satisfactory concentration-dependent change in B/Bo. At this point we tried adding the tracer first so the extract would go immediately into solution. This resulted in a dramatic reduction of the variation at all concentrations, and the decrease in B/Bo was now concentration dependent (Figure 4). The mean B/Bo of the 2 ng/patch samples was greater than 3 SD from the blank, and the means of both the 4 and 5 ng/patch samples were greater than 10 SD from the blank.

At this point, we can only surmise that the high concentration of methanol (75%) in the sweat patch extract may result in a loss of precision for the RIA. Addition of 100 μL of extract to 1 mL of tracer diluted the methanol down to 6.8%. The high variation was probably not apparent in earlier experiments because only 2 or 3 replicates at a concentration were used and they were aliquotted sequentially so that they all were in the Coat-a-Count tube for approximately the same period of time. In all subsequent experiments, the tracer was added to all the tubes before calibrator, control and sample aliquots were added.

Linearity of the cocaine metabolite RIA

The linear range for sweat patch extracts was determined by assay of extracts from 4 to 250 ng cocaine/patch. When the B/Bo was plotted against the log concentration, some curvature became evident at the higher concentrations of 200 and 250 ng/patch (Figure 5). The highest correlation coefficient (r² = 0.991) was achieved with a linear range of 4 to 200 ng/patch. With the four-point logistic curves that were available on the gamma counter software, even higher correlation coefficients were achieved. With 4 ng/patch as the lower calibrator, an r² = 0.996 was found with 150, 200 and 250 ng/patch calibrators at the upper end. We proceeded to use a linear range of 4 to 200 ng/patch and continued to compare the log concentration and 4-point logistic curves because software to prepare the latter may not be available at all sites.

Precision and accuracy of the cocaine metabolite RIA

Replicates of patches at 4, 75, and 150 ng/patch were assayed to determine the precision and accuracy of the assay. Twenty replicates per concentration were tested for the intra-assay ex-
periment. This resulted in % CVs that did not exceed 7.8% and percent targets within 15% (Table II). The mean results of five runs (n = 10 in each run) were used for the interassay experiment. This resulted in % CVs that did not exceed 13.5% and % targets within 13% (Table II).

Detection of heroin, 6-AM, and morphine under normal conditions

After ingestion of heroin, the three main analytes detected in sweat are the parent drug, heroin, and the metabolites 6-AM and morphine (10,18). The RIA and EIA were first assessed, using the procedures of the manufacturer, for their ability to detect heroin, 6-AM, and morphine at concentrations up to 100 ng/patch (Figure 6). The RIA was essentially specific for morphine (Figure 6A). The EIA detected all three analytes well. The calculated cross-reactivities relative to morphine for 6-AM were 42.2 and 50.5% at 10 and 100 ng/patch, respectively. Those for heroin were 27.7 and 32.4 at 10 and 100 ng/patch, respectively. Our values for heroin are similar, and those for 6-AM are slightly higher than reported by Fogerson et al. (26).

Effect of sample volume on opiate RIA

An effort was made to see if increasing the sample volume would increase the sensitivity of the RIA for heroin and 6-AM. Patches at 100 ng/patch of heroin, 6-AM, or morphine were prepared and extracted, and volumes of 25, 50, 100, and 200 µL were assayed (Figure 7). Although there was a sample-volume-dependent decrease in B/Bo for all three analytes, even with a 200 µL sample volume the RIA was still much less sensitive towards heroin and 6-AM than the EIA. Because the EIA met the criteria for detection of all three analytes, and the RIA only detected morphine, further studies were limited to the EIA.

Establishing the LOD and LLOQ for the opiate EIA

As our eventual goal is to compare the quantitative accuracy of the EIA to GC–MS findings in extracts of sweat patches, we decided to use 6-AM as the calibration standard for the EIA. Based on our preliminary findings and a reported LOD of 1.6 ng/patch (26), it seemed reasonable to use the same concentrations as for the RIA LOD and LLOQ experiment. Twenty replicates of patches were fortified at 0, 2, 4, and 5 ng/patch and assayed by EIA (Figure 8).

The patches fortified with 6-AM at 2, 4, and 5 ng/patch had reasonable variance, and a concentration-dependent reduction in signal was clearly evident. The SDblank, however, was too large to establish an LOD or LLOQ based on the samples at 2, 4 and 5 ng/patch that we included in this experiment. Absorbance at the blank – 3 SD was calculated at 24.7 ng/patch using manufacturer's calibrators (Table III). Because this large variation at the blank may be due to some experimental error, we examined data from three other runs where multiple replicates (n = 8) of blank patches were extracted and assayed (Table III). Use of these experiments provided LODs of 1.7 to 2.5 ng/patch and LLOQs of 7.3 to 25.7 ng/patch (Table III).

When we examined the converse situation, the number of SDcontrol that separated controls from the blank (Δ Blank/SD), we found a 2.4 SD separation at 2 ng/patch and greater than 7 SD separation at 4 and 5 ng/patch (Figure 8). These are consistent with a LOD greater than 2 ng/patch and a LLOQ greater than 5 ng/patch.

With the cocaine metabolite RIA it appeared that the presence of the high percentage of methanol in the sweat patch extract contributed to the large variance seen when that assay was run with sample added before the tracer. In the EIA, a buffer solution is added to the well prior to the methanolic sweat patch extract, which would partially dilute the methanol. Attempts to further dilute the methanol by adding the enzyme conjugate prior to sample actually increased the variability (data not shown). Therefore, it did not seem that the presence of methanol was causing the variance in the blanks, and we did not try to further modify the assay. We decided to proceed to establish linearity and see if this apparent variance at the blank would have an impact on the precision and accuracy of the assay.

Linearity of opiate EIA

Linearity was assessed from patches fortified at 4 to 250 ng/patch (Figure 9). Three experiments were run. Both log concentration and 4-point logistic plots were prepared and correlation coefficients ($r^2$) were determined. With the log concentration curves the best correlation (0.987) was found with 4 to 100 ng/patch curves. The 4 to 200, 4 to 250, and 5 to 100 ng/patch curves had $r^2$ of 0.976 to 0.978, while the 5 to 200 and 5 to 250 ng/patch curves had $r^2$ of 0.971. With the 4-point logistic curves, all curves had $r^2$'s that ranged from 0.994 to 0.996. We decided to proceed with a linear range of 4 to 100 ng/patch.

Precision and accuracy of the opiate RIA

Replicate analyses of patches fortified with 5, 25, and 90 ng/patch of 6-AM were used to determine accuracy and precision of the EIA. Intra- and interassay precision was acceptable except for the intra-assay 5 ng/patch calculated using the log concentration curve. Accuracy was good for the 5 and 25 ng/patch controls, but it was consistently more than 30% below target for both intra- and interassay with the 90 ng/patch controls (Table IV). The accuracy and precision of the 5 ng/patch QCs provide evidence for an LLOQ at this concentration rather than the higher range suggested by the preceding experiments. These studies also show that the optimal cutoff
at 25 ng/patch (10 ng/mL of extract) established by Fogerson et al. (26) through receiver operator characteristics is a concentration where the assay is accurate and precise.

Conclusions

When the DPC Coat-a-Count RIA for cocaine metabolite is modified by using 100 µL, rather than 25-µL sample volume and the tracer is added to the tubes prior to sweat patch extracts, cocaine can be accurately and precisely quantitated at concentrations from 4 to 200 ng/patch. Our comparative studies with the STC cocaine metabolite microplate EIA suggests that the RIA gives better sensitivity. This is consistent with the find-

| Table III. Calculation of Opiate EIA LOD and LLOQ from Variance of Blank Patches |
|----------------------------------|------------------|------------------|------------------|
|                              | SD (B/Bo)       | Calculated concentration (ng/patch) |
|                              | N              | Blank – 3 SD    | Blank – 10 SD   |
| 24                             | 0.195          | 24.7            | —               |
| 8                              | 0.037          | 1.7             | 7.3             |
| 8                              | 0.056          | 2.5             | 25.7            |
| 8                              | 0.058          | 2.0             | 24.9            |

| Table IV. Precision and Accuracy of Opiate EIA with Sweat Patch Extracts* |
|-------------------------|------------------|------------------|------------------|
| Target Concentration (ng/patch) | Log concentration curve | Four-point logistic curve |
|                          | Intra-assay       | Interassay       | Intra-assay       | Interassay       |
|                          | Mean              | SD               | % CV             | Mean              | SD               | % CV             | % Target     | Mean              | SD               | % CV             | % Target     | N |
|                          | 5.07              | 1.22             | 24.1%            | 5.07              | 0.51             | 9.6%            | 107.2%        | 5.36              | 0.51             | 9.6%            | 107.2%        | 5 |
|                          | 27.7              | 2.4              | 8.8%             | 26.5              | 3.6              | 13.5%           | 106.0%        | 25.6              | 3.6              | 5.9%            | 106.0%        | 5 |
|                          | 62.4              | 3.8              | 6.2%             | 61.7              | 3.6              | 13.5%           | 68.6%         | 5.54              | 3.6              | 5.9%            | 68.6%         | 5 |
|                          | 5.11              | 0.93             | 18.2%            | 5.54              | 0.52             | 9.3%            | 110.8%        | 22.2              | 2.1              | 6.8%            | 22.2%         | 5 |
|                          | 22.2              | 2.1              | 6.2%             | 23.3              | 1.6              | 6.8%            | 93.3%         | 63.8              | 10.0             | 15.8%           | 63.8%         | 5 |

* Individual concentrations were determined using calibration curves from 4 to 100 ng/mL. Values are from the mean results from 4 different runs where an N of 10 was used in each run, and the intrarun precision and accuracy run where the first 10 replicates were used to determine the mean for interassay determinations.

Figure 7. Effect of sample volume on opiate RIA with analytes at 100 ng/patch. The volumes used (µL) are described in the legend. The comparable results with the opiate EIA are also shown. Results are the mean of two patches.

Figure 8. Variance of 6-AM patch extracts analyzed by EIA. Insert – Δ Blank = the difference between the mean B/Bo of the specified control and the mean of the blanks. Δ Blank/SD = The Δ Blank divided by the SD of the specified control.

Figure 9. B/Bo versus log concentration of cocaine calibrator sweat patches measured by RIA. Values are the mean ± SD of three different experiments.
ings of Spiehler et al. (12) as they reported a LLOQ for the EIA of 32.5 ng/patch (12.5 ng/mL of extract). Based on this difference in sensitivity, we did not evaluate the cocaine EIA for precision and accuracy further and can make no statements on its effectiveness in that regard, although its usefulness as a qualitative screen has been established (12).

The STC Diagnostics microplate EIA for opiates has the cross-reactivity characteristics for heroin, 6-AM, and morphine that suggests its suitability for the detection of heroin use by sweat patch. Although these findings in comparison to the opiate RIA, with or without modifications, drove our experiments to focus on the EIA, they do not preclude the usefulness of the opiate RIA for sweat patch analyses. Further studies are needed in this regard. Precision of the EIA at 25 ng/patch (< 15%) was suitable for use of this concentration as a cutoff, as previously demonstrated (26). Although 5 ng/patch controls did not consistently display suitable precision and acceptable separation from the blank, a concentration between 5 and 25 ng/patch for the LLOQ of the EIA may be found acceptable with further experimentation. We could not establish a linear range for which the EIA gave accurate and precise quantitation; however, our data suggest it would not exceed 4 to 100 ng/patch.

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References


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