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RESEARCH ARTICLE

Measurement of multiple drugs in urine, water, and on surfaces using fluorescence covalent microbead immunosorbent assay

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Abstract

There are a range of applications that require the measurement of multiple drugs such as urine analysis, drug determination in water, and screening for drug contamination on surfaces. Some of the procedures used such as enzyme-linked immunosorbent assay (ELISA) are simple but can only determine one drug at a time, and others such as GC-MS or LC-MS are complex, time-consuming, and expensive. In this study, fluorescence covalent microbead immunosorbent assay (FCMIA) was investigated as a simple method for the measurement of multiple drugs simultaneously in three matrices: diluted urine, water, and on surfaces. Five different drugs of abuse or their metabolites (methamphetamine, caffeine, benzoylecgonine (a metabolite of cocaine), tetrahydrocannabinol (THC), the active ingredient in marijuana, and oxycodone) were studied over the range 0–15 ng/ml. There was no measureable cross-reactivity among the drugs at the concentrations studied. Urine dilutions from 1/50 to 1/2.5 were studied and dilutions less than 1/20 had a significant effect on the methamphetamine assay but limited effects on the benzoylecgonine and oxycodone assays and almost no effect on the THC assay. For assays performed in 1/20 urine dilution, water, and diluted surface sampling buffer, least detectable doses (LDD) were 1 ng/ml or less for the drugs. Surfaces spiked with drugs were sampled with swabs wetted with surface sampling buffer and recoveries were linear over the range 0–100 ng/100 cm^2 surface loading for all drugs. FCMIA has potential to be used for the measurement of multiple drugs in the matrices studied.

Keywords: *Multidrug immunoassay; urine; water; surface sampling*

Introduction

There are a number of areas where measurement of multiple drugs is needed. These include urine analysis for drug-use screening (DHHS SAMHSA 2004) and evaluation of worker exposure to drug residues in the workplace (Connor et al. 2004), determination of drugs in environmental samples such as water (Chiaia et al. 2008), and the determination of surface contamination by drug residues in pharmacies or illicit drug operations (Connor et al. 2004; Martyny et al. 2007). Another possible application is measurement of drugs in serum for therapeutic dose efficacy, which was not addressed in this study (Gamelin et al. 2008). Previously drugs have been determined by simple procedures such as ELISA (DHHS SAMHSA 2005) or by sophisticated and expensive procedures such as GC-MS and LC-MS (Chiaia et al. 2008). While these techniques can provide useful information about the concentration of drugs in the samples, they require that drugs are either measured singly by ELISA or require expensive and time-consuming procedures. Relatively recently a technique called fluorescence covalent microbead immunosorbent assay (FCMIA) for performing multiple immunochemical determinations simultaneously has been developed by the Luminex Corporation (Fulton et al. 1997; Oliver et al. 1998). FCMIA combines several classical methodologies: immunoassays, microspheres, and flow cytometry technology. In FCMIA, immunoassays are performed on solid support microsphere sets with different characteristic internal fluorophores that allow multiple assays to be performed simultaneously on different microsphere sets (multiplexing).

FCMIA has predominantly been used for multiple protein and nucleic acid analytes such as multiple antibodies in serum (Biagini et al. 2003), multiple cytokines in serum

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(Bower et al. 2009), and multiple RNA and DNA viruses in patient samples (Ginocchio et al. 2009). We have been the only group that we are aware of that has applied FCMIA to the measurement of multiple small molecule analytes. In our previous work we applied FCMIA to the measurement of multiple pesticides in water and urine (Biagini et al. 2004). In the work reported in this paper, we investigate the use of FCMIA for the measurement of multiple drugs in urine, water, and on surfaces.

Methods and materials

Chemicals

Standard solutions (1 mg/ml in methanol) for caffeine, tetrahydrocannabinol (THC), oxycodone, and benzoylecgonine were obtained from Cerillant Coporation (Round Rock, TX). The standard solution for methamphetamine, which was 0.1 mg/ ml in methanol, was prepared in-house from pure methamphetamine. Methamphetamine-BSA, benzoylecgonine-BSA, tetrahydrocannabinol-BSA, and oxycodone-BSA conjugates and monoclonal antibodies to methamphetamine, benzoylecgonine, tetrahydrocannabinol, and oxycodone were obtained from Arista Biologicals (Allentown, PA). Caffeine-BSA and the monoclonal antibody to caffeine were obtained from CalBioreagents (San Mateo, CA). All water used was > 18 megohm-cm (Milli-Q Integral, Millipore Corporation, Billerica, MA). Microspheres were obtained from Luminex Corporation (Austin, TX). Activation buffer (0.1 M NaH_2PO_4, pH 6.2), coupling buffer (0.05 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 5.0), wash buffer (phosphate buffered saline [PBS], 138 mM NaCl, 2.7 mM KCl, containing 0.05% Tween20), storage/blocking buffer (PBS, 1% BSA, 0.05% NaN_3, pH 7.4), and HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) were supplied or prepared with reagents supplied by Sigma Chemical Co. (St. Louis, MO). Biotin-labeled anti-mouse IgG, 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide, sodium salt (sulfo-NHS) were obtained from Pierce Biotechnology, Inc. (Rockford, IL). StreptavidinR-phycoerythrin (streptavidin R-PE) was obtained from Molecular Probes (Eugene, OR). The surface sampling buffer was PBS containing 0.1% Triton X-100 (Mallinckrodt Specialty Chemical Company, Paris, KY). The diluted urine was prepared by adding a pre-determined volume of pooled unexposed human volunteer urine to a volume of storage/blocking buffer (1/50 urine = 1 part urine + 49 parts storage/blocking buffer; 1/20 urine = 1 part urine + 19 parts storage/blocking buffer; 1/10 urine = 1 part urine + 9 parts storage/blocking buffer; 1/5 urine = 1 part urine + 4 parts storage/blocking buffer; 1/2.5 urine = 2 parts urine + 3 parts storage/blocking buffer). The 1/3 dilution of surface sampling buffer was made by adding 1 part of the surface sampling buffer and 2 parts of storage/blocking buffer.

Preparation of microspheres

Microspheres were 5.6 μm in diameter and composed of polystyrene, divinyl benzene, and methacrylic acid, which provided surface carboxylate functionality for covalent attachment of biomolecules. Internally, the microspheres were dyed with red and infrared emitting fluorochromes. By proportioning the concentrations of each fluorochrome, spectrally addressable microsphere sets were obtained. The five drug-protein conjugates were coupled to separate unique sets of carboxylate-modified (Luminex) microspheres. After washing twice with activation buffer, five sets of spectrally differentiable carboxylated microspheres (Luminex) were pelleted (5000·g for 2 min) in 1.5 ml centrifuge tubes, sonicated (mini sonicator, Cole Parmer, Vernon Hills, IL), and gently vortexed (vortex Genie, VWR, Intl., West Chester, PA) in 80 μl activation buffer, to which 10 μl activation buffer containing 50 mg/ml EDC and 10 μl of activation buffer containing 50 mg/ml sulfo-NHS were added. The mixture was allowed to incubate for 20 min at room temperature. The activated microspheres were then washed twice in 500 μl coupling buffer, solutions of the five drug-protein conjugates at 40 μg/ ml in 500 μl coupling buffer were added, and the mixture incubated for 2 h at room temperature, with gentle rotating. The coupled microspheres were then washed twice in 1 ml wash buffer, and stored in 0.5 ml storage/blocking buffer. Microsphere concentrations were determined using a microscope and hemacytometer.

Multiplexed analysis

The five drug conjugated microspheres (50 μl), at a working concentration of 1×10^5 microspheres/ml for each microsphere type in storage/blocking buffer were added to wells of a 1.2 μm filter membrane microtiterplate (Millipore Corp., Part #MABVN1250, Bedford, MA) and the liquid aspirated by use of a vacuum manifold filtration system (Millipore, Part #MAVM09601). Mixtures of the five drugs were prepared at eight concentrations (15, 7.5, 3.75, 1.88, 0.94, 0.46, 0.23, and 0 ng/ml) in distilled water, human pooled volunteer urine diluted with a storage/blocking buffer, and surface sampling buffer diluted 1/3 with storage/blocking buffer.

A diluted mixture of drugs (50 μl) were then added to the microspheres in the filter plate from above, and 50 μl of a mixture of the primary antibodies for each drug (anti-methamphetamine, 1/250,000 dilution; anti-caffeine, 1/250,000 dilution; anti-benzoylecgonine, 1:250,000 dilution; anti-THC, 1:100,000 dilution; and anti-oxycodone, 1/75,00 dilution) in storage blocking buffer were added, and then the samples, microspheres, and primary antibodies were allowed to incubate at 37°C (protected from light) for 30 min on a microplate shaker. The wells were washed three times with wash buffer. Biotin labeled anti-mouse IgG (50 μl) in storage/blocking buffer at a concentration of 5 μg/ml was added to the wells and incubated at 37°C for 30 min on a microplate shaker. The wells were again washed with wash buffer and streptavidin R-PE reporter (50 μl), at a concentration of 4 μg/ml, in storage/blocking buffer added to the assay, and the mixture was again allowed to incubate at 37°C (protected from light) on a microplate shaker for 30 min. Finally, the wells were again washed with wash buffer, and the microspheres were resuspended in 100 μl of wash buffer. The plate was shaken vigorously for ~ 1 min to disperse the

microspheres, and was placed into the autosampler platform of the LUMINEX 100 (Luminex) instrument using software, calibration microspheres, and sheath fluid supplied by the manufacturer. The instrument was programmed to collect data from 100 microspheres for each analyte (classified by their internal fluorescence ratio) and acquire the median fluorescence intensity (MFI) of the microsphere-drug conjugate-primary anti-drug conjugate IgG antibody-secondary-anti-IgG-biotin-avidin complex.

Data analyses

Standard curves were constructed from four-parameter logistic-log fits (4-PL, SigmaPlot, SPSS, Chicago, IL) of %B/Bo data (where B = the MFI for each individual drug standard and Bo = the MFI measured for the corresponding blank) vs ng/ml of standard. The least detectable dose (LDD) of the assays was defined as 90% B/Bo, and was interpolated mathematically from the coefficients of the 4-PL equations. Assessment of the 'goodness of fit' and the dynamic ranges of the assays were investigated by evaluating the fit of the standards data to the 4-PL model by 'standards recovery' (Nix and Wild 2001), calculated by evaluating interpolated results from each 4-PL fit (observed concentration) and comparing it to the concentrations of drugs added to the system (the expected concentration) using the following relationship:

$$\%\text{recovery} = \frac{100 \times \text{observed concentration from 4-PL fit of data}}{\text{expected concentration}}$$

The resultant data were analysed for linearity from each drug FCMIA LDD to 15 ng/ml by linear regression. Analyses of recoveries at each dilution were investigated by a Kruskal–Wallis one way analysis of variance (ANOVA, SigmaStat, SPSS, Chicago, IL).

To test for cross-reactivity among the drugs, increasing concentrations of the individual drugs were added to the mixture of methamphetamine, caffeine, benzoylecgonine, THC, and oxycodone microspheres, and anti-methamphetamine, anti-caffeine, anti-benzoylecgonine, anti-THC, and anti-oxycodone added, and the assay was run as above. The percent cross-reactivity was calculated from the following relationship:

$$\%\text{cross-reactivity} = 100 \times \frac{\text{ng/ml @ 50\% B/Bo for heterologous analyte}}{\text{ng/ml @ 50\% B/Bo for homologous analyte}}$$

Recovery of drugs from surfaces

To study the recovery of drugs from surfaces, 10 cm × 10 cm square ceramic tiles (100 cm^2 area) with glazed non-porous surfaces were spiked with solutions of the drugs in methanol and the solution was allowed to dry to produce the desired surface loadings. Surface loadings of 0 ng/100 cm^2, 10 ng/100 cm^2, 25 ng/100 cm^2, 50 ng/100 cm^2, and 100 ng/100 cm^2 were studied. The surface was then sampled using a cotton swab (3-inch cotton tipped applicator; Calapro, Inc., Yorba Linda, CA) wetted in surface sampling buffer. The sampling was done by carefully wiping the surface with the wetted swab in one direction with an overlapping pattern, then repeating the same wiping pattern in a direction perpendicular to the first direction, and finally repeating the original wiping pattern. The swab was then placed in a glass vial containing 1 ml of surface sampling buffer and the swab was extracted. The resulting solution was diluted 1/3 with storage/blocking buffer before analysis as described above.

Results

Cross-reactivity

Figure 1a shows a typical curve produced by the addition of a single analyte (in this case caffeine) to the mixture of beads and antibodies as described in the cross-reactivity experiments. Only the response from the caffeine bead shows inhibition. This is in contrast to Figure 1b, which shows the effect of adding all five drugs simultaneously, where all analyte beads show inhibition. There was no measureable cross-reactivity among the five drugs under the conditions studied.

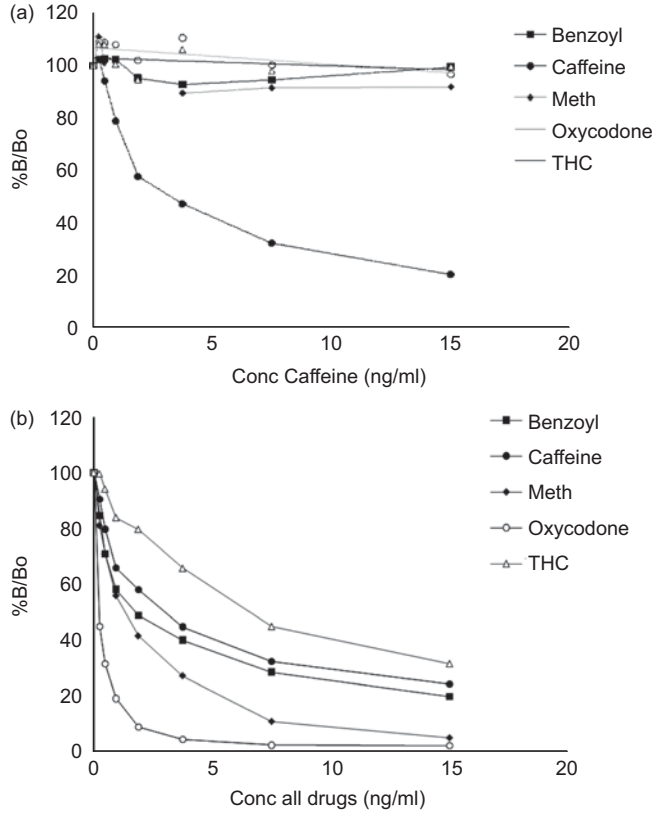

Figure 1. (a) Response of multidrug beads for caffeine addition: Only caffeine was added over a range of concentrations to the mixture of beads for all five drugs. (b) Response of drug beads for addition of all five drugs: All drugs were added over a range of concentrations to the mixture of beads for all five drugs. meth = methamphetamine, benzoyl = benzoylecgonine, %B/Bo = 100 × bead response at a given concentration/bead response at 0 concentration.

Effect of urine on assay

The effect of urine was studied by producing response curves for all drugs at urine dilutions ranging from 1/50 to 1/2.5. The caffeine bead had a large background response to the pooled urine at all dilutions, so the response of the caffeine beads couldn't be studied in urine. The methamphetamine bead showed a large inhibitory effect at urine dilutions less than 1/20 (Figure 2a), while the benzoylecgonine and oxycodone beads showed limited effects and the THC beads showed almost no effect, even at the lowest dilution of 1/2.5. Some component in the urine matrix must inhibit the binding of the methamphetamine antibody with the methamphetamine-BSA conjugate on the bead, but this component has a small effect on the antibodies and beads for the other drugs. However, even the methamphetamine assay showed very similar relative responses at all urine dilutions, as shown in Figures 2b and c. Figure 2b shows that the absolute response as measured by MFI varies greatly at different urine dilutions, while Figure 2c shows that the relative responses as measured by %B/Bo, where B is the response at a given concentration of analyte and Bo is the response at 0 concentration of drug, is relatively constant at different urine dilutions. This shows that methamphetamine in solution shows competitive binding to the methamphetamine antibody, even in the presence of the inhibitory component in the urine matrix. This may mean that the methamphetamine assay could be used at dilutions as low as 1/2.5, as long as the standard curve uses compensation for the urine background; however, this was not studied in this paper. All the remaining urine data for this study was

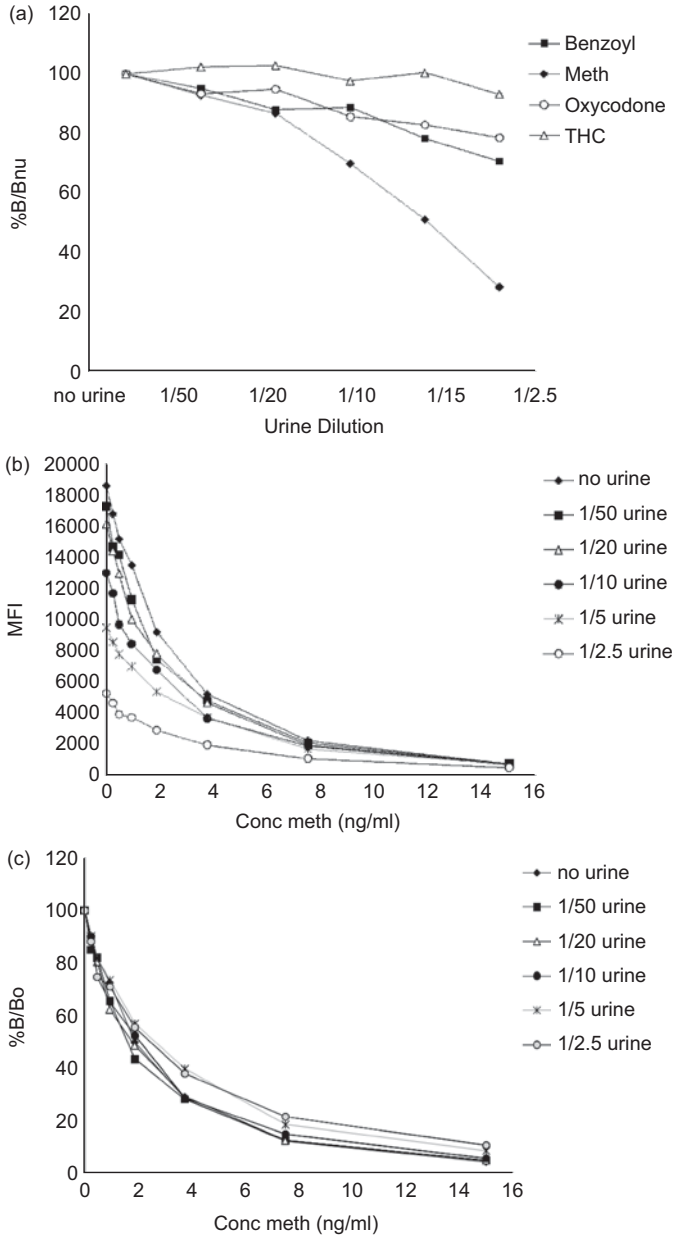

Figure 2. (a) Effect of urine on bead response: The response of the beads was measured at urine dilutions ranging from no urine down to 1/2.5 urine. %B/Bnu = 100 × bead response at a given urine dilution/bead response with no urine. Bead response was measured at 0 drug concentration. (b) Absolute effect of urine dilution on the methamphetamine assay: The absolute response of the methamphetamine assay as MFI over a range of concentrations was measured at urine dilutions ranging from no urine to 1/2.5 urine. MFI = median fluorescence intensity. (c) The relative effect of urine dilution on the methamphetamine assay: The relative response of the methamphetamine assay as %B/Bo over a range of drug concentrations was measured at urine dilutions ranging from no urine to 1/2.5 urine. %B/Bo = 100 × bead response at a given concentration/bead response at 0 concentration.

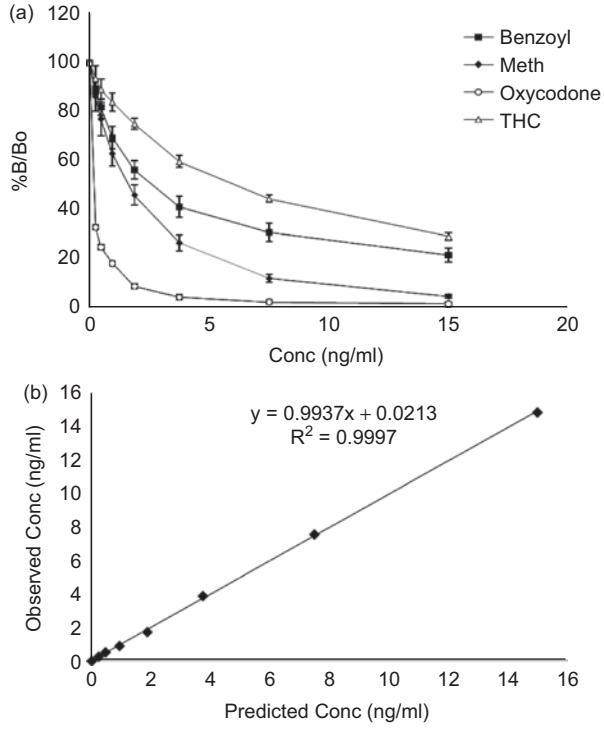

Figure 3. (a) The response of the multidrug assay in 1/20 urine: The response of the assay for methamphetamine, benzoylecgonine, THC, and oxycodone was determined for the range 0–15 ng/ml in 1/20 urine. The error bars represent the standard deviation of the assay response for individual curves taken at different times. (b) Recovery curve for THC: The recovery was calculated by plotting the interpolated results from each 4-PL fit of the THC bead data (observed concentration) against the concentrations of THC added to the system (expected concentration).

taken at 1/20 urine dilution since there was minimal effect on all the assays at this urine dilution.

Response curves and percent recovery in urine

Figure 3a shows the response curves for methamphetamine, caffeine, THC, and oxycodone in 1/20 urine. Using 90% B/Bo as the limit of detection for each drug, the LDD for each drug would be: 0.17 ng/ml for methamphetamine, 0.21 ng/ml for benzoylecgonine, 0.47 ng/ml for THC, and significantly below 0.23 ng/ml for oxycodone. The LDD for oxycodone is significantly less than 0.23 ng/ml since the %B/Bo at 0.23 ng/ml is 32% but the response was not investigated below 0.23 ng/ml. Including the 1/20 dilution correction, the assay LDDs would be 3.4 ng/ml for methamphetamine, 4.2 ng/ml for benzoylecgonine, 9.4 ng/ml for THC, and significantly less than 4.6 ng/ml for oxycodone. As stated above it may be possible to use the assay at urine dilutions lower than 1/20 so the assay LDD could be improved. Recovery was determined as indicated in the Method and materials (plotting the concentration calculated from the 4-parameter fit of the response curve data vs the actual concentration). A typical curve (for THC) is shown in Figure 3b and Table 1 shows the slopes, intercepts, and R^2 values for all the drugs studied. Note that the plot for oxycodone only covers the concentration range below 7.5 ng/ml, since 15 ng/ml had a low %B/Bo.

Response curves and percent recovery in water

Figure 4 shows the response curves for the multidrug assay in water. The LDDs for different drugs were 0.3 ng/ml for methamphetamine, 0.13 ng/ml for caffeine, 0.21 ng/ml for benzoylecgonine, 1.18 ng/ml for THC, and 0.14 ng/ml for oxycodone. Slopes, intercepts, and R^2 of recovery relationships are shown in Table 2.

Response curves and percent recovery in diluted surface sampling buffer

The response curves were determined in a 1/3 dilution of surface sampling buffer for use in determining recovery of drugs from surfaces. Figure 5 shows the response curves for the drugs in this assay and the calculated LDDs are 0.37 ng/ml for methamphetamine, 0.36 ng/ml for caffeine, 0.22 ng/ml for benzoylecgonine, 0.49 ng/ml for THC, and 0.06 ng/ml for oxycodone. Slopes, intercepts and R^2 values of recovery relationships are shown in Table 3.

Recovery from surface samples

As indicated in the Methods and materials, the ceramic tiles were spiked with a mass of each drug and then the surface was wiped with a swab wetted in surface sampling buffer. The resulting solution was diluted 1/3 with storage/blocking buffer and run in the assay. Figure 6 shows the mass of methamphetamine recovered as a function of the mass spiked. Although the recovered mass is ~ 33% of the mass spiked there is a good linear relationship of mass spiked and mass recovered over the entire range of 0–100 ng/100 cm^2. Table 4 shows the linear fits of recovery data for all drugs studied. All

Table 1. Recovery from urine curves. The recovery was calculated by plotting the interpolated results from each 4-PL fit of the bead data for each drug (observed concentration) against the concentrations of drugs added to the system (expected concentration). The table gives the slope, intercept, and regression coefficient for each drug.

Drug	slope	intercept	R^2
methamphetamine	0.9391	0.1467	0.9941
benzoylecgonine	1.0236	−0.0563	0.9984
THC	0.9937	0.0213	0.9997
oxycodone	1.0873	0.072	0.9802

Table 2. Recovery from water curves. The recovery was calculated by plotting the interpolated results from each 4-PL fit of the bead data for each drug (observed concentration) against the concentrations of drugs added to the system (expected concentration). The table gives the slope, intercept, and regression coefficient for each drug.

Drug	slope	intercept	R^2
methamphetamine	0.9836	0.0459	0.9986
caffeine	1.02	−0.0368	0.9979
benzoylecgonine	1.0195	−0.0456	0.9992
THC	0.9822	0.0941	0.9945
oxycodone	0.8991	0.2067	0.9896

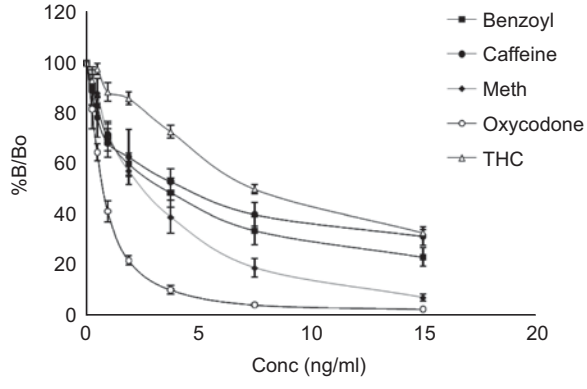

Figure 4. The response of the multidrug assay in water: The response of the assay for methamphetamine, caffeine, benzoylecgonine, THC, and oxycodone was determined for the range 0–15 ng/ml in water. The error bars represent the standard deviation of the assay response for individual curves taken at different times.

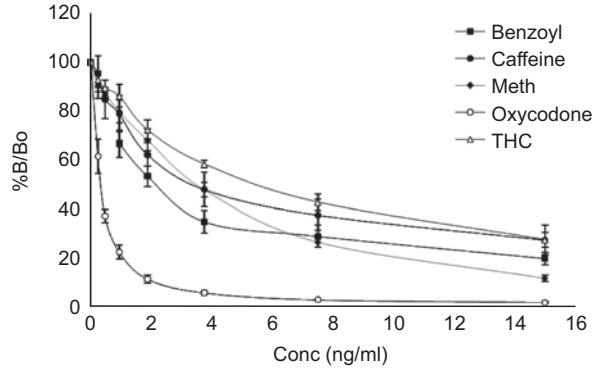

Figure 5. The response of the multidrug assay in 1/3 surface sampling buffer: The response of the assay for methamphetamine, caffeine, benzoylecgonine, THC, and oxycodone was determined for the range 0–15 ng/ml in 1/3 surface sampling buffer. The error bars represent the standard deviation of the assay response for individual curves taken at different times.

Table 3. Recovery from surface sampling buffer curves. The recovery was calculated by plotting the interpolated results from each 4-PL fit of the bead data for each drug (observed concentration) against the concentrations of drugs added to the system (expected concentration). The table gives the slope, intercept, and regression coefficient for each drug.

Drug	slope	intercept	R^2
methamphetamine	0.9728	0.085	0.9971
caffeine	1.0598	−0.1324	0.9944
benzoylecgonine	1.0846	−0.2427	0.9844
THC	0.9968	0.0144	0.9997
oxycodone	1.1593	−0.1798	0.9997

Table 4. Relationship of mass recovered from surface to mass spiked on surface. The recovery of the drug from the surface (ceramic tile) was calculated by plotting the average mass of drug recovered from the surface against the mass of drug spiked on the surface. The table gives the slope, intercept, and regression coefficient for each drug.

Drug	slope	intercept	R^2
methamphetamine	0.3291	0.9655	0.9953
caffeine	0.1655	0.8562	0.9759
benzoylecgonine	0.3578	−0.4868	0.9973
THC	0.1603	0.2401	0.9905
oxycodone	0.3647	−0.4657	0.9975

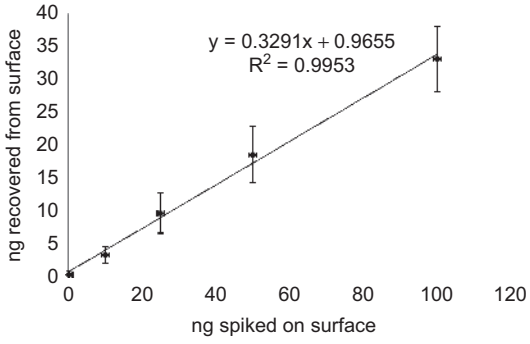

Figure 6. Methamphetamine surface recovery: The mass of methamphetamine recovered from a spiked surface (ceramic tile) was determined by wiping the surface with a swab wetted in buffer, dissolving the methamphetamine on the swab in buffer, and determining the concentration of methamphetamine in the buffer using the multidrug assay. The error bars represent the standard deviation of the recovered mass from the surface for multiple determinations.

drugs showed a good linear relationship of mass recovered vs mass spiked, even though all recoveries are significantly below 100%.

Discussion

There are a number of applications where a cost-effective and simple method for the measurement of multiple drugs in urine, water, and on surfaces could be applied. The assay described in this report shows that it is feasible to use a technique based on FMCIA to satisfy some of these needs.

At 1/20 urine dilution, the assay is sensitive enough to satisfy initial testing requirements for all the drugs measured, which are 50 ng/ml for THC, 300 ng/ml for benzoylecgonine (cocaine and metabolites), 2000 ng/ml for opiates, and 1000 ng/ml for amphetamines (DHHS SAMHSA 2004). If these drugs were present at the cut-off levels, the urine sample would have to be diluted to fall into the range of the assay for quantitative results for several of the drugs. If the assay were used to biologically monitor worker exposure to these drugs through urine analysis then the assay would have to be carried out at a lower dilution than 1/20. This should be possible since only methamphetamine showed a large effect at urine dilutions less than 1/20, but this effect can be compensated for by preparing the standard curve in the proper pooled urine matrix. A standard addition method could also be used to be sure that each individual urine was not different from the pool. Even with this lower dilution, it might not be possible to detect the drugs if they are present at low levels.

The assay can be carried out in water without sample dilution so the assay can be more sensitive. The caffeine levels reported in a sewer effluent study (Chiaia et al. 2008) are capable of being measured with the assay. The samples would have to be diluted considerably for the reported caffeine levels to fall into the quantitative range of the assay. The methamphetamine and benzoylecgonine values reported in the sewer effluent study are within the lower range of range of the assay, while the reported oxycodone levels are generally too low, even though the assay is most sensitive for oxycodone. THC levels were not reported in the sewer effluent study.

For surface contamination, a level of 50 ng/100 cm^2 for methamphetamine (Colorado Department of Public Health and Environment 2005) is the most sensitive suggested level for remediation of contaminated sites. The assay developed in this study can measure down to 10 ng/100 cm^2 for all the drugs studied. Since all drugs can be measured at this level, situations where there is surface contamination by more than one drug can be addressed. The efficiency of surface sampling can be improved by using a different wiping method, but this would likely require a larger volume of surface sampling buffer and therefore would not result in improved detection of contamination since the sample would be diluted further. Different surfaces such as glass, metal, and plastic may have different surface recoveries from the ceramic tiles used in the current study. Also contamination of the surfaces by illicit drug handling or preparation may result in surface loading that has a higher or lower surface sampling efficiency than the surfaces spiked with solutions. These may represent areas for further study. The sampling technique using the swab is simple and convenient. Precise estimation of surface contamination is usually not needed in most circumstances since qualitative evaluation is done to be sure levels are below the threshold. Another possible application being addressed in our laboratory is the measurement of surface contamination by multiple anti-neoplastic drugs (Connor et al. 2004).

Conclusion

The multidrug assay using FCMIA has the potential to be used in a number of applications. It is unlikely that a laboratory would purchase the FCMIA instrument solely for the purpose of measuring multiple drugs. However, as more FCMIA instruments are purchased for the purpose of performing assays

for multiple proteins and nucleic acids, these instruments will also become available to perform multidrug assays. This will increase the number of possible applications for these instruments.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health. Mention of company or product names does not imply endorsement by the National Institute for Occupational Safety and Health.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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