the TPLA test useful for mass screening. We are now studying the possibility of using the TPLA test to monitor syphilitic patients treated with antibiotics and patients with untreated early primary syphilis.

References

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Adsorption Losses from Urine-Based Cannabinoid Calibrators during Routine Use
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The major metabolite of cannabis found in urine, 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (Δ⁹-THC), is the compound most often used to calibrate cannabinoid immunoassays. The hydrophobic Δ⁹-THC molecule is known to adsorb to solid surfaces. This loss of analyte from calibrator solutions can lead to inaccuracy in the analytical system. Because the calibrators remain stable when not used, analyte loss is most probably caused by handling techniques. In an effort to develop an effective means of overcoming adsorption losses, we quantified cannabinoid loss from calibrators during the testing process. In studying handling of these solutions, we found noticeable, significant losses attributable to both the kind of pipette used for transfer and the contact surface-to-volume ratio of calibrator solution in the analyzer cup. Losses were quantified by immunoassay and by radioactive tracer. We suggest handling techniques that can minimize adsorption of Δ⁹-THC to surfaces. Using the appropriate pipette and maintaining a minimum surface-to-volume ratio in the analyzer cup effectively reduces analyte loss.

Indexing Terms: abused drugs · sample handling · variation of source of

Cannabinoids are hydrophobic molecules subject to adsorption to solid surfaces from aqueous solutions such as urine (1). The major metabolite of cannabis found in urine, 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (Δ⁹-THC, 1, see Figure 1), is the principal analyte measured when testing for cannabis use. Most immunoassay methods are calibrated and monitored for quality control with solutions containing this metabolite. When cannabinoid metabolites are lost from the calibrator or control solution through adsorption, the accuracy of the test measurements is affected.

Although commercial preparations of Δ⁹-THC are chemically stable (unpublished data), Δ⁹-THC concentrations in calibrators and controls have been observed to decline during normal use (2). Unused calibrators and controls do not exhibit this decline, suggesting that the loss of drug occurs during use and may be attributable to handling techniques.

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Nonstandard abbreviations: Δ⁹-THC, 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid; Δ⁹-THC, 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid; dpm, disintegrations per minute; TLC, thin-layer chromatography; GC/MS, gas chromatography with mass spectrometry; NIDA, National Institute on Drug Abuse.
Calibrator, control, and sample solutions, all of which contain Δ⁸-THC, come in contact with many surfaces in the course of analysis. Pipettes are generally used to dispense these solutions into sample cups of automated analyzers before testing. Both the inner and outer surfaces of the pipette contact the solution, as do the inner surfaces of the sample cups. Additional contact with surfaces occurs within the chemistry analyzer itself during pipetting, mixing, and incubation. Each time the calibrator contacts a surface, drug adsorption may occur.

The goal of our studies was to discover in which part or parts of the procedure was the loss of cannabinoid occurring; in what amounts; and how to minimize the loss to improve calibrator accuracy. Because samples and calibrators are treated uniformly once they are loaded onto the analyzer, we focused our investigation on the preanalytical portion of the test procedure, i.e., the pipetting of the calibrator into the sample cups. Because this step is typically performed manually, the potential exists for a large degree of variability.

We suspected several areas where preanalytical handling variability could affect the degree of adsorption: the type of pipette used, the technique used in pipetting, the amount of sample transferred to the analyzer sample cup, and time the sample remained on the analyzer before analysis.

We examined three types of liquid-delivery devices commonly used in clinical labs—glass Pasteur pipettes, plastic disposable transfer pipettes, and micropipettes with disposable plastic tips—and observed the drug loss that occurred with each.

In initial studies, we observed that adsorption was quite increased in the large-surface plastic transfer pipettes. This observation led us to conduct studies on the plastic sample cups of three representative analyzers to determine the effect of the surface-to-volume ratio of the cup in cannabinoid loss. We also monitored the time dependence of analyte loss at optimal and nonoptimal calibrator volumes. Using these observations, we developed handling techniques for solutions containing cannabinoids that, when followed, result in more accurate test results.

Materials and Methods

Materials

EMIT Δ⁸ Cannabinoid Calibrators (0, 20, 50, and 100 μg/L; Syva Co., San Jose, CA) were used to calibrate EMIT II Cannabinoid Assays. All calibrator handling studies were done with either EMIT d.a.u.™ Δ⁸ or EMIT Δ⁸ Cannabinoid 50 μg/L calibrators. EMIT Δ⁸ Cannabinoid 50 μg/L calibrators were used for all immunoassay studies except where noted. Radioisotope solutions were prepared in the EMIT Level 0 (negative) calibrator, which contains the same urine matrix as the Δ⁸ Cannabinoid Calibrators. EMIT II Cannabinoid 50 ng Assay reagents (Syva) were used for all automated analyzer experiments. We used the Hitachi 704 (Boehringer Mannheim Diagnostics, Indianapolis, IN), Olympus AU5121 (Olympus Instruments, Lake Success, NY), IL Monarch 2000 (Instrumentation Laboratory, Lexington, MA), and Roche Coas Miras (Roche Diagnostics, Nutley, NJ) instruments to test the EMIT assays. Ultrapure deionized water (Milli-Q; Millipore, South San Francisco, CA) was used for reconstitution and the preparation of buffers.

Sample cups for the analyzers were as follows: Hitachi, 2.5 mL (Elkay Plastics, Shrewsbury, MA); Olympus, 7 mL (Olympus); Monarch, 250 μL (Monarch).

The transfer devices used were DiSPo 13.3 cm (5 1/4 in.) borosilicate glass Pasteur pipettes (Scientific Products, McGaw Park, IL); DiSPo 1-mL graduated plastic transfer pipettes (Scientific Products); and RT-20 and RT-200 pipette tips used with P-200 and P-1000 micropipettes (Rainin Instruments, Woburn, MA).

For many of the radioisotope studies, we used a modified P-1000 Pipetman to control the aspirated volume in the glass Pasteur pipette. A short piece of Tygon® tubing was used to connect the dispensing end of the Pipetman (where the plastic tip would normally fit) to the white plastic base section removed from a 2-mL Pipette Pump™ (Bel-Art Products, Pequannock, NJ). The glass Pasteur pipette was attached to this fitting, and the Pipetman was otherwise used as normal.

Aquasol® liquid scintillation cocktail was used for counting the tritiated tracer on an LC2800 Liquid Scintillation Counter (Beckman Instruments, Brea, CA). An efficiency of 50% was used in calculating disintegrations per minute (dpm) from counts/min.
Preparation of 11-nor-Hexahydrocannabinol-[8,9-3H]-9-carboxylic Acid (2a) and 11-nor-Hexahydrocannabinol-9-carboxylic Acid (2b)

Catalytic reduction was used to incorporate tritium into 11-nor-Δ8-tetrahydrocannabinol-9-carboxylic acid (Δ8-THC; Research Triangle Institute, Research Triangle, NC). A hydrogen-reduced analog, 1b, was also prepared for blending purposes. Axial and equatorial isomers were separated, and only the equatorial isomer was used in the studies. The tritium-labeled and hydrogen-reduced compounds were both prepared under conditions similar to those used by Mechoulam et al. (3).

11-nor-Hexahydrocannabinol-[8,9-3H]-9-carboxylic acid ethyl ester acetate (3a). A solution of 39 mg (0.0933 mmol) of Δ8-THC ethyl ester acetate in 3 mL of ethyl acetate was injected into an evacuated 5-mL septum side-arm flask containing 15 mg of 100 g/kg palladium on carbon. We transferred 11 Ci (0.19 mmol) of carrier-free tritium gas (68 kCi/mol) by Toepler pump into a liquid-nitrogen-cooled flask. After overnight stirring at ambient temperature, the bulk of the volatile activity was removed by distilling about one-half of the solvent to a waste flask. The crude reaction mixture was filtered through a 0.45-μm (pore size) filter. Leachable activity was removed by the filtrate four times from 20-mL portions of methanol. The residue was dissolved in 20 mL of methanol. A radio-scann of the thin-layer chromatographic (TLC) plate (silica gel; ethyl acetate in hexane, 15/85, by vol) revealed an equimolar mixture of the axial and equatorial reduction products. The total activity of the mixture was about 3.5 Ci (32% of theoretical). A portion (88 mCi) of the reduction product was separated by radial chromatography: 1-mm silica gel rotor with a gradient of ethyl acetate in hexane (from 5/95 to 10/90, by vol) at 2 mL/min. Pure fractions (40 mCi axial and 25 mCi equatorial) were retained for deprotection. The specific activity was determined as 22 kCi/mol, based on ultraviolet spectral data (3) and radioassay.

11-nor-Hexahydrocannabinol-[8,9-3H]-9-carboxylic acid (2a). The equatorial product, 3a, was hydrolyzed by stirring in 2 mL of an equimolecular solution of methanol and 1 mol/L NaOH overnight. The reaction was concentrated to one-half volume by rotary evaporation. The resulting solution was extracted with three 4-mL portions of ethyl acetate to remove neutral components. The aqueous phase was acidified and extracted with three 2-mL portions of ethyl acetate. The combined organic phase was washed with 5 mL of saturated sodium chloride and dried over sodium sulfate. The total activity of recovered product was 23.8 mCi (95% yield, >98% purity). The specific activity was presumed to be 22 kCi/mol, the value determined for the ester, 3a.

11-nor-Hexahydrocannabinol-9-carboxylic acid ethyl ester acetate (3b). A solution of Δ8-THC ethyl ester acetate (75 mg) in 5 mL of absolute ethanol was hydrogenated at atmospheric pressure for 25 h in the presence of 14 mg of 50 g/kg palladium on charcoal. The axial and equatorial products were separated on silica gel GF preparative TLC plates (Analtech, Inc., Newark, DE) with hexane/ethyl acetate (21/4 by vol); 20 mg (26% yield) of the axial isomer and 31 mg (41% yield) of the equatorial isomer were recovered. Spectral data for the axial and equatorial isomers were consistent with results obtained by Mechoulam et al. (3) for the corresponding methyl ester.

11-nor-Hexahydrocannabinol-9-carboxylic acid (2b). The equatorial product (3b, 20 mg) was hydrolyzed in an equimolecular solution of methanol/1 mol/L NaOH overnight at room temperature under argon. The reaction was acidified with 3 mol/L HCl, concentrated by rotary evaporation, and extracted three times with 5 mL of ethyl acetate. The organic phases were washed with 5 mL of saturated NaCl, combined, dried over Na2SO4, filtered, and evaporated. Chromatography on silica gel GF preparative TLC plates with chloroform/methanol (9/1 by vol) yielded 10 mg of equatorial 2b.

Surface-to-Volume Ratios of Analyzer Sample Cups

The nonuniform shapes of the sample cups for the automated analyzers were sectioned into regular geometric shapes so that standard formulas could be used to calculate the contact area of the cups as a function of fluid volume. Formulas for the Hitachi, Olympus, and Monarch sample cup dimensions were developed by using combinations of cones, cylinders, and hemispheres. Key measurements of each cup were taken. We created a spreadsheet, using Microsoft Excel to calculate the results.

Calibrator Studies by Immunoassay Methods

Initial studies were done with immunoassay techniques. The observations from the studies can be correlated with effects seen in clinical laboratories during ordinary testing. Follow-up studies were done with the tritiated cannabinoid tracer. These studies were intended to confirm the immunoassay results and to confirm the adsorption of the cannabinoid to the surfaces encountered.

Effect of sampling device and frequency on recovery of cannabinoid in calibrator. For this study, we used EMIT d.a.u. Cannabinoid 50 μg/L calibrators (3-mL bottles). Aliquots of 100 μL were transferred from the original calibrator bottle into Cobas Mira sample cups, with use of a fresh transfer device—glass Pasteur pipettes, a disposable tip micropipette, and single-use plastic transfer pipettes—for each transfer. Each device was used to sample a fresh bottle of calibrator until the bottle was empty. The glass Pasteur pipette and plastic transfer pipette are not volumetric, so we measured their aliquots by counting drops. Each nonvolumetric device was used to aspirate 1 to 2 mL of solution. The aliquot volume was estimated by counting 3–5 drops, and the residual liquid in the pipettes was returned to the parent calibrator bottle. The micropipettes delivered the 100-μL aliquots exactly, with no remaining liquid to be returned to the original container. The caps of the sample cups were closed, and the pipetted calibrator aliquots were incubated at room temperature for 30 to 60 min to allow the analyte concentration to equilibrate.
between the solution and the cup surface. Each aliquot was assayed in triplicate on a Coas Mira S analyzer.

Relationship between dispensed volume of calibrator in automated analyzer sample cups and immunoassay response. For the Hitachi 704, the Olympus AU5121, and the Monarch 2000 analyzers, we pipetted a series of various calibrator volumes into the appropriate sample cups with a glass Pasteur pipette and assayed after equilibration for 30 min (covered, at room temperature). Volumes were estimated visually and confirmed by counting drops. At least five aliquots of each volume were prepared and tested with each analyzer.

Effect of incubation times. About 1 mL of calibrator was pipetted with glass Pasteur pipettes into Hitachi 704 sample cups. The aliquots were tested either immediately or after a 5-min delay. The delay incorporated by the analyzer in reaching the first sample in the batch, ~2 min, was additional delay time.

Adsorption Studies with Tritium-Labeled Analyte

Preparation of cannabinoid tracer solution. Separate solutions of the unlabeled and tritiated cannabinoid derivatives (2b and 2a) were prepared in EMIT Calibrator Level Ø for the radioisotope studies. Each solution contained a total cannabinoid content of 50 µg/L. These were blended to provide a final test solution with a scintillation counting response that had a high signal-to-noise ratio. The cannabinoids were added to the urine matrices from 10 mg/L stock solutions of 2a and 2b in ethanol, and the cannabinoid solution was handled in glass at all times. A new tracer solution was prepared for each experiment.

Effect of multiple sampling of cannabinoid solution with various transfer devices. The cannabinoid tracer solution (2a and 2b) was divided into 3.5-mL portions in 10-mL glass bottles. These were repeatedly sampled with the three types of pipettes. The three types of transfer devices were used to pipette 30 successive 100-µL aliquots from each bottle into Hitachi cups. A fresh device was used for each transfer, and the aliquots were allowed to equilibrate for 30 min afterwards. A disposable-tip micropipette was then used to transfer 50 µL of each pipetted aliquot from the sample cup into liquid scintillation fluid for counting.

Relationship between sample cup volume and analyte recovery. The radioisotopic cannabinoid solution (2a) was dispensed with a glass Pasteur pipette into Hitachi, Olympus, and Monarch sample cups. A modified Pipetman P-1000 was used to control the dispensed volume in the glass Pasteur pipette. The cups were gently rocked for 30 min to simulate the processing movements of the automated analyzers. A micropipette was used to transfer 50 µL of sample into scintillation fluid for counting. The concentration of recovered cannabinoid was calculated.

Time dependence of cannabinoid adsorption in analyzer sample cups determined by radioisotope recovery. The tritiated cannabinoid tracer solution (2a) was transferred by 100-µL and 300-µL aliquots into Monarch cups. Glass Pasteur pipettes were used with a modified Pipetman P-1000 to control the dispensed volume. At various time intervals, 50 µL of solution was transferred into liquid scintillation fluid and counted.

Measurement of analyte adsorbed to sample cups by radioisotope recovery. A glass Pasteur pipette in a modified Pipetman P-1000 device was used to dispense 100-µL aliquots of cannabinoid tracer solution (2a) into Monarch sample cups. After incubation at room temperature for 30 min, 50 µL of each sample was pipetted into liquid scintillation fluid and counted. The remainder of the solution was aspirated from the cup, and the cup was rinsed three times with a buffer solution (0.5 mol/L sodium acetate, 0.5 mol/L NaCl, pH 5.0) to remove liquid residue. Previous studies have shown that Δ⁹-THC has very low solubility in this solution (unpublished work), so the last drops of tracer solution could be removed from the cup without affecting the amount of analyte adsorbed to the walls. The cup was then rinsed three times with 300-µL aliquots of ethanol to extract the adsorbed cannabinoid. The combined ethanol rinses and the combined buffer rinses were counted separately in liquid scintillation fluid.

Results

Transfer Devices

The results of the multiple sampling studies are shown in Figure 2. In both studies, an approximately linear relationship exists between aliquot number and recovery of analyte throughout the greater part of the calibrator bottle. There may be a greater rate of loss as the last aliquots are removed. The immunoassay method shows clear distinctions among the three devices; the plastic transfer pipette retains the greatest amount of analyte and the glass Pasteur pipette the least amount. The radioisotope study also shows that the plastic transfer pipette adsorbs the greatest amount of analyte. The glass Pasteur pipette and the micropipette appear to adsorb cannabinoids to a similar extent. For both studies the initial recovery with the glass Pasteur pipette is greater than that for the other two devices.

The slope of the regression lines for the plastic transfer pipette is obviously different from the other two slopes in both studies. It is not obvious that the slopes of the regression lines for the glass Pasteur pipette and micropipette are different. If the slopes are different for both studies, then the results appear anomalous. The immunoassay results suggest that the glass Pasteur pipette adsorbs less cannabinoid than the micropipette, and the radioisotope study suggests that the micropipette adsorbs less. To clarify this point, we compared these slopes by analysis of covariance. The final points in each data set were omitted to remove any "last draw" artifacts from the calculation; thus, we used points through aliquot 25 in the immunoassay study, and through aliquot 26 in the radioisotope study. The slopes of the two regression lines for the immunoassay study are significantly different (P = 0.006); however, the two slopes are not significantly different for the radioisotope
The approximate loss in concentration can be calculated from the radioisotope study results. Comparing the first and last aliquots taken by each device gives the maximum amount of loss. The glass Pasteur pipette and the micropipette showed a similar maximum concentration loss of 4.5 µg/L, or 9% of the initial concentration. In contrast, the plastic transfer pipette lost 14 µg/L, 28% of the initial concentration. If the last points are omitted, as in the statistical analysis, then the glass Pasteur pipette and micropipette lost about 2 µg/L and the plastic transfer pipette 6 µg/L.

Dispensed Volume and Analyte Recovery

The surface-to-volume ratios for the three sample cups were calculated as described above, based on measurements taken from the sample cups and geometric approximations. For clarity, an example of the calculation is given here.

The Hitachi sample cup can be divided into three regular geometric sections. A small hemisphere at the bottom supports a truncated cone, which is topped with a cylindrical section. A volume of 600 µL fills this cup into the cylindrical section. Direct measurement of the dimensions of the hemispherical section shows that it holds 12 µL and has a surface area of 0.20 cm². The conic region will hold an additional 545 µL, given its height and side angle. The conic section has a surface area of 2.97 cm². The remaining 43 µL of sample extends 4.97 mm into the cylindrical section, which adds another 0.16 cm² of surface area. The total surface area in contact with the sample is 0.20 + 2.97 + 0.16 = 3.33 cm², which gives a surface-to-volume ratio of 5.55 cm²/µL (3.33 cm² + 600 µL). The same type of calculation was made for all three cups for a range of volumes.

The results of the studies of surface-to-volume ratio in the automated analyzer sample cups are shown in Figure 3. In all cases, the liquid volume in the analyzer cup affects the recovery of the cannabionid from solution. The radioisotope study was used to confirm the relationship seen between surface-to-volume ratio and analyte recovery. The range for this study was limited to low volumes, for which the change in surface-to-volume ratio would be largest.

The total change in recovery was calculated from the radioisotope study results. For the Hitachi cup, the recovery change was 2 µg/L, whereas the Monarch and Olympus cups showed 9 µg/L and 8 µg/L changes, respectively. In general, the relationship of recovery to volume correlates inversely with the surface-to-volume ratio of the cup.

Time Dependence of Adsorption

On the Hitachi 704 the response of 1 mL of the cutoff calibrator (50 µg/L) with no additional time delay was 387 ΔA/min (SD 2.92 ΔA/min). The response after a 5-min delay was 389 ΔA/min (SD 1.22 ΔA/min). The radioisotopic time study with Monarch sample cups is summarized in Figure 4. In this case, two pipetted volumes, 100 µL and 300 µL, are compared. The lower volume shows a strong time-dependence for analyte recovery. Recovery does not plateau until 10 min after dispensing the solution. The higher pipetted volume is not dependent on delay time, because the amount of analyte adsorbed to the sample cup is small relative to the total amount present.

Recovery of Analyte Adsorbed to Solid Surfaces

The analyte lost from solution was accounted for by the quantity of analyte extracted from the plastic surfaces with ethanol. Table 1 summarizes the results.

Discussion

By examining the behavior of cannabionid solutions subjected to common laboratory use, we have demonstrated that nonoptimal handling of calibrator and cont-
The optimal concentration results for EMIT immunoassay (C) and radioisotope tracer study (E) are compared with the calculated surface-to-volume ratio (Δ) for each analyzer cup. The immunoassay rates are not directly related to concentrations (refer to text for explanation). Arrows indicate the recommended sample volume for each analyzer cup. A schematic drawing of each analyzer cup is included, with the optimal volume indicated: (A) Hitachi sample cup (capacity = 2000 μL); (B) Monarch sample cup (250 μL, size, capacity = 500 μL); (C) Olympus sample cup (capacity = 7000 μL). The stock tracer solution had an activity of 1.18 × 10^8 dpm/mL (Hitachi and Olympus studies) or 1.20 × 10^6 dpm/mL (Monarch study).

Fig. 3. Effect of volume in sample cup on recovery of cannabinoids: results for EMIT immunoassay (C) and radioisotope tracer study (E) are compared with the calculated surface-to-volume ratio (Δ) for each analyzer cup. The immunoassay rates are not directly related to concentrations (refer to text for explanation). Arrows indicate the recommended sample volume for each analyzer cup. A schematic drawing of each analyzer cup is included, with the optimal volume indicated: (A) Hitachi sample cup (capacity = 2000 μL); (B) Monarch sample cup (250 μL, size, capacity = 500 μL); (C) Olympus sample cup (capacity = 7000 μL). The stock tracer solution had an activity of 1.18 × 10^8 dpm/mL (Hitachi and Olympus studies) or 1.20 × 10^6 dpm/mL (Monarch study).

Loss was lowest with the glass Pasteur pipette. After ~30 samples were removed, the analyte recovery was still >95% of its initial value. The micropipette with disposable plastic tips gave similar recovery. Use of plastic disposable transfer pipettes led to the greatest amount of analyte loss. The results of the two methods in Figure 2 show the same trends for the types of pipette, especially when comparing the glass Pasteur pipette and micropipette with the plastic transfer pipette.

Another indicator of adsorption loss is the initial recovery. The initial recovery is the immunoassay rate (dpm) for the first aliquot in each series. Because the calibrator bottles start at the same concentration, differences in initial recovery are directly related to the adsorption from the cannabinoid calibrator aliquot to the inside surface of the transfer device. Each aliquot encounters this adsorption loss as it is transferred. In contrast, the slope of the line is produced by cannabinoid adsorption from the solution remaining in the bottle. In both studies, the glass Pasteur pipette has a greater initial recovery than either of the two plastic devices.

The three types of pipettes studied differ both in material and in geometry. The glass and plastic transfer pipettes have similar configurations but are constructed of different materials. Therefore, one can conclude that the construction material is a critical component in terms of Δ^9-THC lost through adsorption. This conclu-
sion is consistent with previous work; glass is reportedly an optimal material for the handling of hydrophobic cannabinoids (1).

The disposable tip micropipette, although plastic, has some advantages over the glass Pasteur pipette. Only the volume to be transferred is aspirated, so the parent solution is exposed to the external surface area of the pipette tip only as the tip is inserted into the solution. This is an improvement over nonvolumetric transfer devices, which expose the parent solution repeatedly to the internal surface area of the pipette. When a nonvolumetric device is used, excess solution is aspirated. After delivery of the desired volume the remaining solution is returned to the parent container, decreasing the concentration of analyte in the parent solution.

Fresh pipettes and tips were used for each sampling in the study to simulate typical conditions. Normally, a calibrator solution would be sampled infrequently for calibration and the pipette would not be stored for repeat use. Multiple sampling and the use of new pipettes effectively expose the solution to a large surface area. The exposure of the solution to this surface area causes progressive loss of the analyte.

The comparison of sample cups with different geometries demonstrates that recovery differences are also due to the surface-to-volume ratio in the cup. An inverse relationship exists between surface-to-volume ratio of the cups and the recovery of analyte remaining in solution. The Hitachi 704, Monarch 2000, and AU5121 were chosen for study because they are representative of the automated analyzers used in clinical laboratories. Each represents a distinct analyzer architecture. Although the immunoassay results have been reported as rates instead of concentrations (see explanation above), the trend of increasing rate with increasing calibrator volume is directly comparable to the concentration results of the radioisotope study.

The surface-to-volume ratios of the sample cups are greater at low volumes, when the surface of the sample is close to the bottom of the cup. The recovery of Δ⁹-THC is least when the liquid level is near the bottom of the cup. In this region, the surface area of the bottom of the cup is large in comparison with the surface area of the side. As the sample volume increases, the contact surface area of the sides increases. Eventually the additional surface area of the bottom of the cup is negligible in comparison with the side surface, and the surface-to-volume ratio plateaus. Analyte recovery mimics this change in surface-to-volume ratio, reaching a plateau at a similar volume. The optimum volume of liquid dispensed into analyzer cups brings the liquid level in the cup to a region with a low surface-to-volume ratio. In most analyzer cups this means that the optimal volume is in a cylindrical section above a conical or hemispherical bottom portion.

For all three sample cups studied there is good correlation between the immunoassay and radioisotope methods. The apparent difference between the two in the study of Hitachi sample cups is due to the small total variation in recovery. The range of recovery in the radioisotope study, 0.04 × 10⁶ dpm/mL, accounts for only 4% of the total analyte in solution, or 2 µg/L in a 50 µg/L stock solution. The other two sample cups had a greater range of recovery, 16% for Olympus cups and 18% for Monarch cups. The Hitachi sample cup also has a smaller surface-to-volume ratio, 5 cm²/mL, than the other two sample cups (7 cm²/mL for Olympus cups and 10 cm²/mL for Monarch cups). This supports the inverse relationship between surface-to-volume ratio and cannabinoid recovery.

The time for equilibration of the cannabinoid calibrator in the cup is not a critical variable when the cup is filled to the appropriate volume. In sample cups with less than optimal fill volumes, loss of analyte is time-dependent and plateaus at 10 min. This time-dependence is further evidence that the effect of sample cup fill volume on analyte recovery is due to the interaction of the analyte with the cup surface, and does not originate from interactions with the transfer device or the analyzer itself. The effect of the transfer device on drug recovery is separate from the effect of fill volume.

The extraction of analyte adsorbed to the Monarch analyzer cup accounts for the analyte loss from solution. This observation is consistent with those previously made regarding the hydrophobic nature of the analyte and its affinity for nonpolar surfaces (1, 4).

These observations led to the development of the following guidelines for handling cannabinoid calibrator solutions in immunoassay applications. By following these guidelines, one can obtain greater accuracy for cannabinoid test results:

- Glass Pasteur pipettes or micropipettes with disposable plastic tips should be used rather than disposable plastic transfer pipettes for dispensing calibrator solutions. Concern for sharp objects in biohazardous waste may make the micropipette the best choice for clinical laboratories. Loss of analyte during sampling is minimized by use of glass or micropipettes, but care should still be taken to avoid excess contact between the cannabinoid solution and other surfaces.

- Calibrator volume sufficient to minimize the surface-to-volume ratio should be dispensed. Recommended sample cup fill volumes for the three sample cups studied are shown in Figure 3. Hitachi cups should be filled with 600 µL of calibrator, Olympus cups with 1000 µL, and Monarch cups with 300 µL. The optimal volume for sample cups for other automated analyzers can be easily determined by using any of the methods described above.

- The time of equilibration of calibrator in the analyzer cup is not a critical variable when the optimal volume is used.

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The potential of three types of separator materials found in conventional blood-collection tubes for interference in therapeutic drug measurements was assessed. None of the separators (based on acrylic, silicone, or polyester polymers) had any significant effect on the concentrations of seven drugs (theophylline, digoxin, phenytoin, phenobarbital, gentamicin, ethanol, and cyclosporine) in blood specimens that were processed and analyzed promptly. Storage of specimens for 24 h resulted in an average 2.4% increase in theophylline values in specimens collected in tubes with the acrylic separator (P = 0.024); an average 8.1% decrease in phenytoin in specimens collected in tubes with the polyester-based separator (P < 0.001); and an average 4.2% decrease in phenobarbital in specimens collected in tubes with the polyester-based separator (P = 0.02). All other drug concentrations were not significantly affected. A small decrease in phenytoin (7.9%; P < 0.01) was seen when the specimen volume in 7-mL tubes containing polyester-based separator was reduced to 1.0 mL; all other drug concentrations were unaffected by partial filling of tubes. Paired blood specimens from pediatric patients, when collected in plain tubes and tubes containing acrylic separator, yielded no significant differences for theophylline, digoxin, tobramycin, phenytoin, or phenobarbital concentrations. The three commercially available separators had only small effects on therapeutic drug concentrations, and a newly developed separator based on an acrylic resin was suitably inert.

Indexing Terms: analytical error · variation, source of

Although evacuated tubes have been used successfully in the collection of blood specimens for routine laboratory tests for many years, it is important to recognize that components of these collection devices can, in some instances, interact with collected specimens to alter test results in undesired ways. Blood-collection tubes have become sophisticated devices in response to the needs of phlebotomists and laboratory personnel to increase efficiency and decrease the potential for infection through handling. These improvements have resulted in polymeric materials being introduced into components of the tubes that come in contact with the specimen, and have increased the potential for absorption of analytes or contamination of the specimen. Some recent studies focusing on the interactions of these specimens with blood analytes concluded that test results may be altered in specimens collected in these new types of containers (1–11). Such tubes contain a polymeric material that has a density intermediate between that of serum or plasma and blood cells and that forms a layer on top of the packed cells during centrifugation. Of particular concern in these studies was the potential for this material to interact with hydrophobic drugs. The concentrations of such drugs in specimens stored for extended periods might be artifically decreased as a result of interaction with the polymeric barrier, and measurements would thus be clinically misleading (2–5, 9–11). These effects were accentuated in specimens stored in tubes containing a polyester-based separator gel (Becton Dickinson SST) for 6–72 h prior to analysis (2–4, 10, 11). Others reported that tubes containing silicone-based separator material (Monoject Corvac or Samplette) had less pronounced effects on drug concentrations (2, 5, 9), but small changes in some drug concentrations were observed. The availability of a newly formulated separator (acrylic polymer) for Monoject Corvac evacuated tubes prompted us to compare therapeutic drug concentrations in blood specimens collected in tubes already in widespread use with concentrations in blood collected in tubes containing the new acrylic separator. Of particular interest were the effects of specimen storage on drug

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