Differences in Binding Affinities of MDA, MDMA, MDEA, Amphetamine, Methamphetamine, and their Deuterated Analogues to Solid-Phase Extraction Cartridges

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Abstract

This study evaluated the potential for partial separation of drugs from their deuterated internal standards using Cerex® Polycom™ CLIN II solid-phase extraction (SPE) cartridges. After elution from the column and derivatization, gas chromatography–mass spectrometry results showed that the target compound eluted from the SPE cartridge prior to its deuterated form. This elution separation effect was greater for 3,4-methylenedioxy-methamphetamine (MDA) and methamphetamine (MAMP) than for the other drugs studied. When the drugs were eluted in 0.5 mL increments from a 50 mg sorbent bed, no drug appeared in the first fraction. The drug to internal standard ratios (expected value 1.00) for subsequent fractions collected were 1.30, 1.07, and 0.83 for MDA/MDA-d5; 1.65, 1.18, 0.67, and 0.56 for MDA/MDMA-d5; and 1.37, 1.18, and 0.95 for MDEA/MDEA-d6. For d-AMP and d-MAMP, the expected ratio was 0.40. The subsequent ratios were 0.63, 0.46, 0.35, and 0.34 for d-AMP/d-AMP-d11; and 1.00, 0.59, 0.25, and 0.18 for d-MAMP/d-MAMP-d11. The affinity of d-MAMP-d14 was shown to be greater than that of d-MAMP-d5, and deuteration at the propyl end of the molecule was shown to increase binding more than deuteration on the phenyl group.

Introduction

Amphetamine (AMP) and methamphetamine (MAMP) are stimulants of the central nervous system (1). Their designer methylenedioxy analogues, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) are Schedule I drugs that cause the user to experience euphoria, sociability, and a sense of general well-being (2,3). Because of their addictive nature and adverse health effects, illegal use of these drugs by members of the United States Armed Forces is detrimental to military readiness, due to compromised service member health and mission performance.

An effective approach to deter the use of illicit drugs, including AMP, MAMP, and their designer methylenedioxy analogues in the workforce is to monitor employees and military personnel through random drug testing (4,5). Forensic analysis of urine specimens for these amphetamines by the Department of Defense (DoD) (6,7) or Department of Health and Human Services guidelines (8) requires initial immunoassay test(s) followed by confirmation and quantification by gas chromatography–mass spectrometry (GC–MS).

Accurate quantification of AMP, MAMP, MDA, MDMA, MDEA, and other drugs of abuse has been achieved by the use of deuterated internal standards that have chemical and physical properties that are almost identical to that of the parent drug. The only difference between the drug and its deuterated internal standard is that one or more hydrogen atoms has/have been substituted with deuterium. MS is ideally suited for identification and quantification because the drug and internal standard can easily be distinguished from one another by the increased mass resulting from the substitution of deuterium for one or more hydrogens.

Although the use of deuterated internal standards is the method of choice in most forensic urine testing laboratories, some limitations have been reported. MS fragmentation can include (M – H2)n processes that generate a series of “cluster ions”, where n is the number of hydrogens involved in the process. Deuterated internal standards can also include (M – Dn) processes that are likely to generate ions interfering with the intensities of some of the ions attributed to unlabeled drug (9,10). Additionally, ionization efficiencies of a drug and its deuterated analogue may differ because of differences in concentrations and retention times. These effects would not be expected with other isotopically labeled analogues such as 13C internal standards (10).

In order to perform quantitative analysis by GC–MS or other analytical methods, the drug and internal standard were ex-
tracted from a sample matrix using procedures such as liquid–liquid and solid-phase extraction (SPE) (11,12). Various studies have reported the success of SPE applied to the analysis of many drugs, including AMP, MAMP, and their methylenedioxy derivatives (13–20).

There are several commercially available brands of SPE columns with different resin beds that have been used for extraction of amphetamines (13,14,21). Selecting the appropriate SPE column depends upon the volume of the specimen, the specimen matrix, the sensitivity of the analytical method and the characteristics of the analyte. The Cerex Polycrom CLIN II SPE columns evaluated in this study are mixed mode with a hydrophobic highly cross-linked divinylbenzene (DVB) polymer and strong cation exchanger in the form of bound sulfonic (SO₃⁻) groups. This column does not need pre-conditioning, and the small 10-µm particle size provides sufficient surface area for drug binding in a 35- or 50-mg resin bed.

The purpose of the current study was to conduct comparative elution analysis of AMP, MAMP, MDA, MDMA, and MDEA and their respective internal standards using strong cation-exchange SPE Cerex Poly Crom CLINII columns with a 50-mg resin bed. Experiments were devised to challenge the assumption that amphetamine-like drugs and their internal standards are eluted equally from SPE columns. Results from this study can help in determining the appropriate elution volume and extraction procedures for eluting drugs of abuse from SPE columns. It was not the intention of this study to provide a comprehensive comparison of SPE columns from alternate vendors, but rather to assess the general elution characteristics of amphetamine-like drugs on Cerex Polycrom CLINII columns.

**Experimental Procedures**

**Materials**

Methanol, ethyl acetate, hydrochloric acid, sodium periodate, sodium hydroxide, ammonium hydroxide, and dibasic potassium phosphate were all ACS grade purchased from Fisher Scientific (Fairlawn, NJ). The derivatizing reagents, heptafluorobutyric anhydride (HFBA) and R-(-)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (R-MTPA), were obtained from Sigma Aldrich-Fluka (St. Louis, MO). Stock solutions of d-AMP and d-MAMP and racemic solutions of MDA, MDMA, AMP-d₆, AMP-d₁₁, AMP-d₃ (ring), AMP-d₃ (sidechain), MAMP-d₅, MAMP-d₁₄, MDA-d₅, and MDMA-d₅ were purchased from Cerilliant (Round Rock, TX). The structures of these drugs are shown in Figure 1. d-AMP, d-MAMP, MDA, MDMA, and MDEA controls, samples and standards were prepared in certified drug-free urine from Roche Diagnostics (Indianapolis, IN). Cerex Polycrom CLIN II with 50 mg (6-mL capacity) of sorbent were purchased from SPEware (Baldwin Park, CA).

**Extraction**

All extraction steps were done using Speedisk® 48 positive-pressure extraction manifolds (SPEware, San Pedro, CA) and polymer-based cation exchange/mixed-bed extraction columns (Cerex Polycrom CLIN II, 50-mg resin bed), as described by Klette et al. (22).

Samples were prepared by pipetting 2.0-mL aliquots of standards or controls containing MDA, MDMA, and MDEA into 15-mL glass centrifuge tubes (Kimble Chase, Vineland, NJ). A 100-µL aliquot of internal standard solution containing 0.01 mg/mL each of MDA-d₅, MDMA-d₅, and MDEA-d₆ and 0.75 mL of 2.3 M phosphate buffer (pH 9.0) was added to each sample. In a similar manner, 100 µL of internal standard solution containing 0.01 mg/mL each of AMP-d₁₁ and MAMP-d₁₄, 1.0 mL of 2.3 M phosphate buffer (pH 9.0), and 3.0 mL of 0.1 M sodium periodate were added to 2.0 mL of urine sample that contained AMP and MAMP. All samples were vortex mixed for 5 s. Specimens that were treated with periodate were capped and incubated for 10 min at room temperature. Periodate oxidation prevents potential interference from over-the-counter medications containing pseudoephedrine and ephedrine which may be present in urine specimens (23). All specimens were centrifuged at 2000 rpm for 5 min to remove any precipitate.

**Figure 1.** Chemical structures of the deuterated drugs used in this study.
Samples were then poured into 50 mg Cerex Polycrom CLIN II SPE columns and placed onto the Speedisk® apparatus. A low nitrogen flow of 2–3 standard cubic feet/h (SCFH) was applied to load the columns. Next, 2.0 mL of distilled water was applied to the columns followed by 2.0 mL 0.1M HCl (For MDA, MDMA, and MDEA) or 0.1M acetic acid (for AMP and MAMP). Nitrogen gas (2–3 SCFH) was applied after addition of each reagent and the columns were dried for 2–3 min at 25 psi. The columns were then washed with 2.0 mL of methanol and 2.0 mL of ethyl acetate. Nitrogen gas (2–3 SCFH) was applied after the addition of each reagent and the columns were dried for 2–3 min at 25 psi. A solution of ethyl acetate/ammonium hydroxide (98:2) or methylene chloride/acetone/triethylamine (80:20:2) was used to elute AMP and MAMP, whereas a solution of ethyl acetate/methanol/ammonium hydroxide (80:20:2) was used to elute MDA, MDMA, and MDEA. The target compounds were eluted by gravity flow either directly into 1.5-mL capacity autosampler vials (ALS) vials for MDA, MDMA, and MDEA, or into 15.0-mL conical tubes for AMP and MAMP. Fractions with 0.5 mL elution volume were incrementally collected until the drugs had fully eluted from the column. In some cases, a low nitrogen flow of 2–3 SCFH was applied for the collection of the first 0.5 mL fraction. After addition of 0.050 mL of 1% concentrated HCl in methanol to each eluate, the eluates were evaporated to dryness.

Derivatization

MDA, MDMA, and MDEA samples were derivatized by adding 0.035 mL HFBA and 0.100 mL ethyl acetate to the ALS vials that were then capped, mixed and incubated for 10 min at 60–70°C. Samples were removed from the heating block, allowed to cool, and then evaporated to dryness at 50–60°C under a stream of nitrogen. Samples were reconstituted in 100 μL of ethyl acetate for GC–MS analysis. d-AMP and d-MAMP samples were derivatized by adding 20 μL of R-MTPA solution (500 mg R-MTPA in 8.0 mL acetonitrile) and 200 μL chlorobutane/triethylamine (100:7.5) to the 15.0-mL collection glass tubes. Samples were vortex mixed, capped, and incubated in a heating block at 60°C for 30 min, at which time 0.100 mL anhydrous ethanol was added. Samples were vortexed for approximately 15 s and then reincubated in a heating block for 30 min at 60°C. Tubes were removed and evaporated to dryness at 30°C under a stream of nitrogen. Samples were reconstituted in 200 μL of ethyl acetate prior to transfer to ALS vials for GC–MS analysis.

Analysis

All samples were analyzed by GC–MS under selected ion monitoring (SIM) mode using an Agilent Technologies (Palo Alto, CA) 6890N GC and a 5973N mass selective detector. GC injections were performed with a 7083 automated liquid sampler. Drug to internal standard ratios were calculated directly from the area abundance printed on the chromatograms.

MDA, MDMA, and MDEA method

GC separations were performed using an Agilent J&W (Wilmington, DE) DB-5ms bonded-phase capillary column (15 m × 0.25-mm diameter, 0.25-μm film thickness). The injection port temperature was 245°C. Initial oven temperature was maintained at 150°C for 0.10 min then ramped at 30°C/min to 250°C and held for 1 min. All injections were performed with a split ratio of 12:1 using helium as a carrier gas and a column flow rate of 0.6 mL/min. The transfer line temperature was 295°C.

AMP and MAMP method

GC separations were performed using an Ultra 1 (methyl siloxane bonded-phase) capillary column (25 m × 0.200-mm, 0.33-μm film thickness). The injection port temperature was 220°C. GC separations were performed under isothermal conditions with an oven temperature of 210°C. Samples were injected under split mode using a split ratio of 10:1 using helium as a carrier gas and a column flow rate of 0.9 mL/min. The transfer line temperature was maintained at 280°C.

Table I. Summary of Assay Parameters

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ions (m/z)</th>
<th>Quantitation Ion</th>
<th>Qualifier Ion Ratios</th>
<th>Confirmation Cutoff (mg/mL)</th>
<th>Drug/IS Ratio (Theoretical)</th>
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<tr>
<td>AMP</td>
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<td>260</td>
<td>118/260, 162/260</td>
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<td>–</td>
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<tr>
<td>AMP-d6†</td>
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<tr>
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<td>258, 394</td>
<td>258</td>
<td>394/258</td>
<td>–</td>
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</tbody>
</table>

* Used when analyzed with AMP-d6.
† Used for relative binding studies, not used in quantitative assays.
All quantifications were made using Agilent MSD ChemStation version E01 software. Analyte concentrations for specimens and controls were determined by single-point calibration at the current DoD cutoff. For d-AMP and d-MAMP, the calibration standard contained 100 ng/mL of each drug. The MDA, MDMA, and MDEA calibration standard contained 500 ng/mL of each drug. Table I shows the ions and ion ratios used for analysis.

**Results and Discussion**

Because the drug to internal standard ratio is the basis for quantification, recovery of both the analyte of interest and its corresponding deuterium labeled internal standard during the extraction process is critical. Elution profiles show the amount of drug eluting off of the column versus volume. The internal standard and drug should elute from the SPE cartridge at the same time or in the same proportion in order to have consistent recoveries and quantification. Differences in the binding of the drug and internal standard to the column resin have the potential to produce quantification errors if all of the drug and internal standard are not eluted uniformly from the column.

**MDA, MDMA, and MDEA**

In order to assess the elution profiles during SPE, urine specimens containing 500 ng/mL MDA, MDA-d$_5$, MDMA, MDMA-d$_5$, MDEA, and MDEA-d$_6$ (Figure 1) were applied to Cerex Polycrom CLIN II SPE columns with a sorbent bed size of 50 mg and eluted in 0.5-mL increments. Ten 0.5-mL fractions were collected, but over 99% of the drugs were eluted in the first six or seven fractions. Aliquots containing less than 1% of drug are not shown in the figures. After derivatization and analysis by GC–MS, the fraction of drug and internal standard eluted in each aliquot was calculated from the ion abundances of the quantitative ions for the drug and corresponding internal standard (Table I). The theoretical ratio of drug to internal standard (DI ratio) is 1.00; however, the actual DI ratio may be greater or less than unity because of the actual concentration of the starting material and any experimental error in the procedure. Differences in DI ratios between the collected 0.5-mL fractions indicate non-uniform elution and, therefore, differences in partitioning of the drugs between the column sorbent and the elution solvent.

The DI ratios for MDA/MDA-d$_5$, MDMA/MDMA-d$_5$, and MDEA/MDEA-d$_6$ (Figures 2–4, respectively) were greater than unity for the early fractions and less than unity in the later fractions, indicating that the drug is eluted prior to its deuterated analogue. For example in Figure 2, a ratio of 1.30 for MDA/MDA-d$_5$ revealed that the second 0.5-mL fraction contained 57% MDA and 43% of MDA-d$_5$. The third 0.5-mL fraction from this column had a ratio of 1.07 corresponding to 52% of MDA and 47% of MDA-d$_5$. If elutions were uniform, the DI ratios of these fractions would be unity with each analyte present at 50%. Both analytes were fully eluted with 2.5 mL of elution buffer with a cumulative DI ratio of 1.12. Similarly, MDEA and its internal standard exhibited elution profiles analogous to those of MDA and MDA-d$_5$ (Figure 3). Again, early fractions favor drug elution over internal standard and the cumulative DI ratio after 2.5 mL of elution buffer was 1.16.
In contrast, MDMA shows an even more pronounced difference in elution profile from its internal standard than for MDA or MDEA (Figure 4). The early fractions again contain more drug, and the latter contain more internal standard. Specifically, the successive fractions had DI ratios above three and below unity, corresponding to 62%, 56%, 45%, and 36% MDMA for fractions 2–5, respectively. More elution buffer was required for full elution of analytes with a cumulative DI ratio of 1.07.

The difference in binding affinity between MDMA and the other two designer amphetamines implicates the methyl- and ethyl- groups bound to the amine as significant to such interactions. Because the apparent order of elution does not follow expected trends in increased hydrophobicity imparted by these substituents and proportional interaction with the same phase actions, Be cazuthe apparent order of elution does not follow expected trendsin increased hydrophobicity imparted by these substituents and proportional interaction with the same phase actions.

MDA MP-d 14 w as applied to 50-mg C erexPolycrom CL INIISPE Amphetamineand methamphetamine GC–MS, the fraction of drug and internal standard eluted in parallel with increases in the number of substituents on the amine or their relative sizes. Consequently, the observed affinities are most likely due to perturbations in basicity of the amines, where the inductive effect from the amino methyl group causes MDMA to be more basic than MDA, but the binding of MDEA is reduced compared to MDMA due to a steric interaction of the larger amino ethyl group. For all of the drugs, deuteration caused higher affinity for the packing material as discussed later, but the binding of MDMA-d 3 was additionally increased because of the proximity of the deuteriums to the nitrogen.

Amphetamine and methamphetamine

Urine specimens containing d-AMP, AMP-d 11 , d-MAMP, and MAMP-d 14 were applied to 50-mg Cerex Polycrom CLIN II SPE columns and eluted with 0.5-mL aliquots of ethylacetate/ammonium hydroxide (98:2). After derivatization and analysis by GC–MS, the fraction of drug and internal standard eluted in each aliquot was calculated from the ion abundances of the quantitative ions for the drug and corresponding internal standard (Table I). The theoretical DI ratio was 0.40 (100 ng/mL drug to 250 ng/mL internal standard) with any deviation indicating non-uniform elution and, therefore, differences in binding affinities to the column sorbent.

As with the designer amphetamines, the drugs and internal standards were present in different proportions in successive fractions and the methyl analogue eluted later than nonmethylated compounds, presumably due to the previously discussed differences in the affinity of each drug to the column packing material (Figure 5). d-AMP eluted before d-AMP-d 11 , and d-MAMP eluted before d-MAMP-d 14 , indicating that the deuterated forms of the drug have a greater affinity to the column packing material. After five fractions, the cumulative DI ratio for d-AMP/d-AMP-d 11 was 0.43 and that for d-MAMP/d-MAMP-d 14 was 0.40.

When the polarity of the elution solvent was changed, there were still differences in the elution profiles between the drug and its corresponding internal standard. Using methylene chloride/acetonetriethylamine (80:20:2) as the elution solvent caused the drugs to elute later and increased the separation between the drugs and their deuterated analogues compared to ethylacetate/ammonium hydroxide (98:2). With methylene chloride/acetonetriethylamine (80:20:2), 8 fractions were required to collect 99% of d-AMP and d-AMP-d 11 , and 10 fractions were required to collect 99% of d-MAMP and 97% of d-MAMP-d 14 (data not shown).

Degree and position of deuterium substitution

Molecules where hydrogen has been replaced by deuterium have been shown to have slightly different properties compared to the non-deuterated molecules (25). The C-H bond has a higher vibrational frequency, a longer bond length and is weaker compared to the C-D bond. The shorter C-D bond length results in a more compact size imparted by the shorter C-D bonds compared to C-H bonds (26). These properties can cause small but measurable differences in binding affinities of the drug and its deuterated internal standard. Cody et al. (11) reported gas-phase separation between an analyte and its deuterated analogue using HP-1, HP-5, and DB-17 GC columns, and concluded that the observed separations were correlated to the amount of deuteration.

To investigate the effect of the number of deuterium atoms on the binding of AMP and MAMP to Cerex Polycrom CLIN II SPE columns, the binding of two other deuterated drugs, AMP-d 6 and MAMP-d 5 was investigated. Spiked urine specimens containing d-AMP, d-MAMP, AMP-d 6 , AMP-d 11 , MAMP-d 5 , and MAMP-d 14 were applied to 50-mg Cerex Polycrom CLIN II SPE
columns and eluted with 0.5-mL fractions of ethylacetate/ammonium hydroxide (98:2), as shown in Figures 6 and 7. d-AMP eluted before d-AMP-d₆ and d-AMP-d₁₁ and the drugs eluted in the following order: d-AMP > d-AMP-d₆ > d-AMP-d₁₁ > d-MAMP > d-MAMP-d₁₄. Relative binding was calculated using the d-AMP-d₁₁/d-AMP-d₆ ratio as measured by the 130/125 abundance ratios and d-MAMP-d₁₄/d-MAMP-d₆ as measured by the 281/278 abundance ratios. No drugs were eluted in the first 0.5-mL aliquot. For the second through sixth fractions, the internal standard ratios were 0.89, 0.94, 0.92, 0.88, and 0.76 for d-AMP-d₁₃/d-AMP-d₆ and 0.79, 0.90, 1.13, 1.28, and 1.36 for d-MAMP-d₁₄/d-MAMP-d₆. The increasing d-AMP-d₁₃/d-AMP-d₆ ratio with each successive fraction indicates d-MAMP-d₁₄ has a greater affinity to the packing material than d-MAMP-d₆. The pattern was less apparent for d-AMP-d₆ and d-AMP-d₁₁; however, the d-AMP-d₁₁/d-AMP-d₆ ratio was reduced for the last fraction which contained 1.5% of the drug.

These data indicate that analyte affinity for the column resin increases with increasing deuteration and more so for methamphetamine.

Although the difference of nine deuteriums between d-MAMP-d₆ and d-MAMP-d₁₄ resulted in a lower affinity of the former for the column material, the difference of five deuteriums between d-AMP-d₆ and d-AMP-d₁₁ did not cause a significant change in binding properties. The smaller effect of deuteration substitution on d-AMP may be related to the position of the deuteriums as well as to the number of substitutions. The only difference between d-AMP-d₆ and d-AMP-d₁₄ is the five deuteration substitutions on the phenyl ring. In comparison, the differences between d-MAMP-d₆ and d-MAMP-d₁₄ include the five deuteration substitutions on the phenyl ring, one deuteration on the beta carbon, and three deuterations on the methyl group attached to the alpha carbon. Taken together, these data indicate that deuteriums either enhance the basicity of the proximal amine through increased induction from the higher electron density imparted by the shorter C-D bond length, or enhance hydrophobic affinity of the propyl moiety, with aryl interactions being less significant. That the former enhancement to basicity is more significant is supported by the greater discrimination between d-AMP and d-MAMP, and MDA and MDMA, due to induction from the methyl group, as discussed previously.

Several studies (11,25,27–29) have shown that, in general, deuteration of hydrocarbons will produce a more polar molecule, but if heteroatoms such as N, O, or S are present, deuteration may either increase or decrease the hydrophobicity depending upon the position of the deuteration substitution (25). Mráz et al. (27) found that, if deuteration was bound to the formyl group of N,N-dimethylformamide, N-methylformamide, or formamide, the deuterated forms eluted later than the unlabeled molecules, indicating the deuteration of the formyl group caused the molecule to be more lipophilic than the unlabeled molecule (27). In a similar manner, deuteration of MDA, MDMA, MDEA, AMP, and MAMP near the amine would be expected to cause the deuterated drug to be more lipophilic than the non-deuterated drug.
To investigate the effect of the position of the deuteriums on binding affinity, two different AMP-d5 internal standards were analyzed using the fractionation method described in this study. d-AMP-d5 (ring) has five deuteriums on the phenyl ring, and d-AMP-d5 (side chain) has five deuteriums on the propyl moiety as shown in Figure 1. If deuteration on the side chain is more influential in binding of the drug to the column material compared to deuteration on the ring, then d-AMP-d5 (ring) would be expected to elute before d-AMP-d5 (side chain). Figure 8 shows the elution profile of urine specimens containing 500 ng/mL of AMP-d5 (ring) and 500 ng/mL of AMP-d5 (side chain) from a 50-mg CEREX Polycrom CLIN II SPE that were eluted sequentially with 0.5-mL aliquots of ethyl acetate/ammonium hydroxide (98:2). The ratios of d-AMP-d5 (side chain)/d-AMP-d5 (ring) as measured by the 264/260 abundance ratios increased from 0.73 to 1.13 for the second through the fourth fractions before leveling off at 1.17 and 1.15 for fractions five and six. The data show that d-AMP-d5 (ring) is eluting earlier and indicate that the binding of d-AMP-d5 (side chain) is increased due to increased electron density on the nitrogen from the deuteriums on the propyl moiety.

Quantification errors
Separation of drug and internal standard during SPE is a potential source for inaccurate quantification of drug for a sample of unknown concentration. As long as the same amount of elution solvent is used for all analytical runs, the elution disparity is not expected to cause issues, because the DI ratio will be uniformly disparate for all specimens. However, for a scenario where drug elutes before its internal standard, insufficient elution solvent used to establish a DI ratio upon calibration will lead to all subsequent quantifications being underestimated. In contrast, if less elution solvent is used for unknowns, relative to that used for the calibrator, quantities will be overestimated. The data in Table II illustrate this latter scenario, wherein insufficient volumes of the elution solvent ethyl acetate/ammonium hydroxide (98:2), were added to an SPE column containing a sample of 100 ng/mL of d-AMP and 100 ng/mL of d-MAMP relative to a calibration standard eluted with 2.0 mL of the same solvent.

Figure 9 shows the relative recovery of d-MAMP and d-MAMP-d14 as a function of elution volume. Using 1.50 mL (i.e., 0.50 mL less than was used for the calibration standard) did not affect the DI ratio, but when the elution volume was reduced to 1.00 mL, there was a 12% and 36% increase in the quantification of d-AMP and d-MAMP, respectively. This increase was due to a change in the DI ratio because of the differences in elution profiles between the drug and internal standard. These types of errors can be avoided by accurate addition of elution solvent to all of the calibrators, controls, and specimens in the analysis. Additional assurance can be achieved by using a procedure that uses sufficient excess elution solvent where small changes of elution solvent do not affect quantification or to use a procedure that has a minimal deuterium isotope effect.

### Table II. Quantification of a 100 ng/mL Sample d-AMP and d-MAMP from a 50-mg Resin Bed CEREX Polycrom CLIN II SPE Column with Different Volumes of Ethyl Acetate/Ammonium Hydroxide (98:2)*

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>1.00</td>
<td>112 ± 3</td>
<td>136 ± 9</td>
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<tr>
<td>1.25</td>
<td>104 ± 0.3</td>
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<td>102 ± 0.4</td>
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</tbody>
</table>

* The internal standard was 250 ng/mL of d-AMP-d11 and d-MAMP-d14.

### Conclusions
The work described herein shows that the different binding affinities and solubilities of deuterated drug and drug produce different elution profiles for MDA, MDMA, MDEA, d-AMP, and d-MAMP and MDA-d5, MDMA-d5, MDEA-d5, d-AMP-d5, d-MAMP-d5, and d-MAMP-d14. Deuteration of these basic drugs increased the affinity of the drug for the SPE packing material. This difference is most likely due to an increase in basicity of the amine imparted by deuteration. The difference in binding between drug and deuterated drug was greater when there was a deuterated methyl group on the amine. The relative difference in binding of drug and its deuterated analogue to DVB mixed phase SPE columns may result in quantification errors if disproportionate amounts of drug and internal standard are left on the SPE column. Adequate recovery of drug and internal standard for analysis can be achieved by applying sufficient elution solvent to assure that essentially all of the drug and internal standard are eluted from the SPE column.

Because commercial SPE columns differ in their sorbent characteristics, particle size, packing, and physical characteristics, validation procedures should evaluate elution volume and elution profiles of all analytes to assure that disproportionate losses of drug and internal standard are not occurring during the extraction process. Further study in this area with...
different deuterated analogues or $^{13}$C analogues, which would not be expected to have a significant inductive effect, would be beneficial in characterizing the binding of drugs to mixed phase and other column sorbents.

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