# A Matter of Mass

**Kevin F. Foley,[a,*] Nicholas V. Cozzi ,[b] and Paul F. Daley[b]**

## CASE DESCRIPTION

A urine sample was submitted to our laboratory for drugs of abuse testing. The ordering provider mentioned that he was specifically interested in assessing the presence of 3,4-methylenedioxymethamphetamine (MDMA). Screening was performed with an automated immunoassay (Abbott Alinity) for opiates, oxycodone/oxymorphone, benzodiazepines, ethanol, THC, cocaine, barbiturates, methadone, and amphetamine/methamphetamine. The only component that screened positive was amphetamine/methamphetamine. The positive amphetamine result reflexed confirmatory testing. Confirmatory testing was performed using enzyme hydrolysis followed by analysis using a SciEx Triple Quad 5500 with a Kinetex, 2.6 um Biphenyl 100 Å, LC Column 50 × 3.0 mm. Confirmatory testing with LC-MS/MS revealed a possible trace amount of the MDMA qualifier ion at the expected retention time but did not meet criteria to justify a finding of MDMA (qualifier and quantifier ions for MDMA are 194.1/163.1 and 194.1/105.1, respectively). The presence of a peak corresponding to the retention time of MDMA, as well as the positive amphetamine screen result, led to further inquiry of the ordering provider as to the origins of the specimen.

## CASE DISCUSSION

The specimen in question came from an outside clinical facility from a patient who was part of a drug development study. The drug used in the study consisted of MDMA with 3 deuterium atoms on the *N*-methyl moiety (MDMA-d3). Substitution of deuterium for hydrogen atoms has the potential to affect the pharmacokinetics of a drug (1). In this case, researchers were interested in determining whether the deuterated compound had a longer pharmacodynamic profile due to the kinetic isotope effect, as has been reported for other agonists and substrates (2). Although rare and expensive to produce, isotopic drugs have been used historically in clinical pharmacology testing (3). Substitution of deuterium for hydrogen can also render a drug novel with regard to the patenting process.

Upon further discussion with the ordering provider, it was revealed that the patient in question had orally ingested 75 mg of MDMA-d3. The urine had been collected 5 hours after dosing. The Abbott Alinity immunoassay reports that MDMA will produce a result that is approximately equivalent to the cutoff calibrator, when MDMA is present in at least 0.8 ug/mL, when the 500 ng/mL cutoff is used (4). While this concentration is 1.6 times higher than that needed for d-amphetamine

[a]Kaiser Permanente Northwest, Portland, OR, 97031, United States; [b]Alexander Shulgin Research Institute, Lafayette, CA 94549, United States.
*Address correspondence to this author at: 13705 NE Airport Way, Portland, OR, 97230 United States. E-mail kevin.f.foley@kp.org.
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| Ion | Signal Intensity | Internal Standard | Retention Time (expected 0.493-0.603) | Retention Time Pass? | M2/M1 (expected 1.3) | Ratio Pass? |
|---|---|---|---|---|---|---|
| MDMA 1 | 1665 | 9080945 | 0.542 | Yes | 0.490 | No |
| MDMA 2 | 816 | 9080945 | 0.502 | Yes | 0.490 | No |

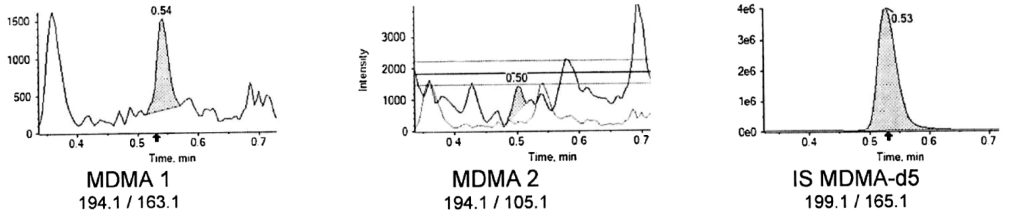


**Fig. 1. LC-MS/MS findings. The *y* axis represents signal intensity; the *x* axis is time in minutes. Arrows refer to expected retention time for MDMA. Although a small amount of the MDMA 1 ion appears to be present, it was very low in intensity; neither MDMA 1 or MDMA 2 ions were detected.**

(and thus the assay is somewhat less sensitive for the detection of MDMA when compared to amphetamine or methamphetamine), the urine concentration of MDMA-d3 in the sample was still sufficient to cause a positive screen result.

When confirmatory testing with LC-MS/MS was performed, the qualifier and quantifier ions for MDMA (194.1/163.1 and 194.1/105.1, respectively), could not be clearly detected. A slight peak was seen for MDMA 1 that corresponded to the expected retention time for MDMA (Fig. 1). However, the signal intensity of this peak was orders of magnitude lower than would commonly be seen with a positive sample, and thus this peak was considered background "noise." It is possible that this peak represents a trace amount of nondeuterated MDMA that may be a contaminant present in the synthesis of the MDMA-d3.

Our method requires that an m/z ion ratio must be within 15% of expected values to pass scrutiny. Our MDMA confirmation test employs multiple reaction monitoring. Multiple reaction monitoring achieves its high sensitivity and specificity via utilization of a quadrupole mass spectrometer that first targets the ion corresponding to the compound of interest, followed by subsequent fragmentation of that target ion to produce product ions. These product ions are then used for quantitation purposes. Only compounds that meet both criteria, i.e., they contain the specific parent ion and the specific product ions that correspond to the mass of the molecule of interest, are isolated within the mass spectrometer. While the deuterated MDMA in the urine had solubility properties that would be identical to MDMA and thus would pass retention time criteria, the molecular weight of the MDMA-d3 was 3 atomic mass units heavier. Thus, the product ions M1 and M2 did not have the appropriate m/z ratio to qualify as MDMA. Since our internal standard for the LC-MS/MS assay uses MDMA-d5 and not MDMA-d3, the ingested MDMA-d3 isotope did not confound the internal standard.

## TAKEAWAYS

- Positive drug screens should be reflexed to mass spectrometry for confirmation.
- MDMA is not always detected with immunoassays used to screen for amphetamine/methamphetamine, but if concentrations of



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MDMA are high enough, positive results can be obtained.
- Deuterated substitution of compounds increases the molecular mass, which results in different ion ratios that will not be detected with conventional multiple reaction monitoring assays.
- Although rare, isotopic drugs are sometimes used in research settings.
- Follow-up with providers or patients can provide key information when drug screen results are discordant with confirmatory findings.

**Author Contributions:** *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

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