National Laboratory Certification Program

DRUG TESTING MATTERS

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Methamphetamine Enantiomers: Proficiency Testing Results and Analysis Enhancements

Part 2

This is the second of a two-part Drug Testing Matters series. The first article, issued in January 2013, included a review of methamphetamine chiral chemistry, background information on Medical Review Officer (MRO) interpretation of methamphetamine results, and a study summary of methamphetamine purity in nasal inhalers and commercial standard materials. This article provides a summary of laboratory testing capabilities based on a focused proficiency testing (PT) set and recommendations to enhance methamphetamine enantiomer testing in federally regulated workplace programs.

Introduction

The *Medical Review Officer Manual* (1) from the Department of Health and Human Services (HHS) includes guidance for interpreting methamphetamine enantiomer test results as follows: "If there is greater than 80% L-methamphetamine, the results are considered to be consistent with over-the-counter (OTC) use. If there is more than 20% D-methamphetamine present, the results indicate the use of some source other than the OTC product, and the result is verified as positive. This is a very conservative interpretation."

Revisions to the HHS *Mandatory Guidelines for Federal Workplace Drug Testing Programs* (HHS Guidelines) in 2010 included lowering the methamphetamine confirmatory test cutoff from 500 to 250 ng/mL (2). Subsequently, some Medical Review Officers (MROs) reported an increase in the number of methamphetamine positive results with D-methamphetamine <20%. In some of these cases, the total methamphetamine concentration was >5,000 ng/mL.

Methamphetamine Enantiomers: Proficiency Testing Results and Analysis Enhancements

The Substance Abuse and Mental Health Services Administration (SAMHSA) directed studies by RTI International (RTI) to evaluate the testing capabilities of HHS-certified laboratories and to reassess the current HHS interpretation guidance (3).

A search of the literature found several reports of urine concentrations of L-methamphetamine following Vick's inhaler use; however, none were performed in recent years. In one 1993 study, four subjects followed the manufacturer's directions (inhale through each nostril every 2 hours) and used the medication for 5 days (4). The urine concentrations were 250 ng/mL or less in the urine collected over the 5-day study. In the same report, three individuals using double the daily dose (inhale through each nostril every hour) for 3 days obtained the highest concentrations of L-methamphetamine: 1390, 1290, and 740 ng/mL in urine collected on the second and third days. L-amphetamine, a metabolite of L-methamphetamine, was not detected in any of the urine specimens. In a 1988 report of more excessive doses, three subjects were instructed to take several deep inhalations every 20 minutes for 6 hours (5). The peak urinary L-methamphetamine concentrations for each of the three subjects were 6000, 1950, and 1520 ng/mL in urine collected in intervals over 24 hours. In these subjects, L-amphetamine with levels up to 455 ng/mL was present.

RTI designed studies to (1) determine the amount of methamphetamine enantiomers in OTC nasal inhalers and (2) determine the purity of commercial methamphetamine enantiomer standards. The study results verified the manufacturers' stated content of the nasal inhalers and standard solutions, but also showed a high bias in enantiomer results obtained using the chiral derivatizing agent employed by most HHS-certified laboratories. (See the January 2013 *Drug Testing Matters* article.)

After completing these studies, a methamphetamine enantiomer proficiency testing (PT) set was prepared for HHS-certified laboratories. The PT samples were designed to assess the amphetamines immunoassays' cross-reactivity to L-methamphetamine at different concentrations and to determine the analytical capabilities of HHS-certified laboratories that conduct methamphetamine enantiomer testing. PT data and conclusions are summarized below.

RTI's studies enabled an evaluation of the current HHS guidance (i.e., >20% D-methamphetamine) for identifying non-OTC methamphetamine use, and the development of recommendations for enantiomer testing in federally regulated workplace programs.

Proficiency Testing Set

The PT set consisted of 18 samples designed to determine the lowest concentration and lowest percentage of D-methamphetamine that HHS-certified laboratories could accurately quantify. The target concentrations ranged from 0 to 1,000 ng/mL D-methamphetamine (lowest spiked concentration of 25 ng/mL) and from 0 to 4,750 ng/mL L-methamphetamine (lowest spiked concentration of 75 ng/mL). These samples provided challenges containing D-methamphetamine percentages that ranged from 0% to 100%, with emphasis on 5% to 20% (i.e., 11 of the 18 samples).

Laboratory Testing

The HHS Guidelines require laboratories to use immunoassay methods for initial drug testing. For amphetamines, the initial test cutoff is 500 ng/mL, with D-methamphetamine as the target analyte. The immunoassay reagents used at the time of the PT analysis are shown in **Table 1** below.

For confirmatory drug testing, the HHS Guidelines require laboratories to use a method combining chromatographic separation and mass spectrometric identification. The required confirmatory test cutoff for amphetamine and methamphetamine is 250 ng/mL. The HHS Guidelines allow, but do not require, methamphetamine enantiomer testing to assist MROs in the interpretation of methamphetamine test results. At the time of the studies, 24 of 37 HHS-certified laboratories performed methamphetamine enantiomer testing. All 24 laboratories used gas chromatography/mass spectrometry (GC/MS) for amphetamine confirmatory analysis. For methamphetamine enantiomer testing, the 24 laboratories used GC/MS with a chiral derivatizing reagent. Twenty-three laboratories used N-trifluoroacetyl-L-prolyl chloride (L-TPC) and one laboratory used (R)-(-) methoxytrifluoromethylphenylacetic acid (MTPA) as the chiral derivatizing reagent for enantiomer testing.

The 24 laboratories tested all PT samples using their amphetamines initial test, amphetamines confirmatory test, and D- and L-methamphetamine enantiomer test.

Immunoassay Study

As noted above, one purpose of the PT set was to assess the amphetamines immunoassays' cross-reactivity to L-methamphetamine at different concentrations. **Table 1** lists the five immunoassays used by the participating laboratories.

Table 1. Amphetamines Initial Test Reagents Used for PT Analysis

Number of Laboratories	Reagent Manufacturer	Reagent Name
17	Siemens	EMIT®II Amphetamines
3	Thermo Scientific	CEDIA® DAU Amphetamines
2	Roche Diagnostics	ONLINE DAT Amphetamines (KIMS)
1	Thermo Scientific	DRI® Amphetamines Assay
1	Thermo Scientific	CEDIA® Amphetamine/Ecstasy Assay

Note: EMIT = Enzyme Multiplied Immunoassay Test; CEDIA = Cloned Enzyme Donor Immunoassay; KIMS = Kinetic Interaction of Microparticles in Solution.

One PT sample was prepared to mimic higher urine concentrations that might result from excessive nasal inhaler use. The PT sample contained 1,000 ng/mL L-methamphetamine and 150 ng/mL L-amphetamine (i.e., a metabolite of L-methamphetamine). Sixteen of the 17 laboratories using the Siemens EMIT® II amphetamines reagent obtained a positive immunoassay result. Laboratories using other reagents obtained negative results for this sample.

A PT sample containing 4,000 ng/mL of L-methamphetamine and 150 ng/mL of L-amphetamine tested positive at all 17 laboratories using the EMIT® II reagent and at the single laboratory using the CEDIA® Amphetamine/Ecstasy reagent.

The immunoassay data reveal that the reagent used by the majority of HHS-certified laboratories (Siemens EMIT® II Amphetamines reagent) cross-reacts with L-methamphetamine. Therefore, use of OTC nasal inhalers may cause immunoassay-positive results.

Quantification Study

The principle objectives of this study were to determine the lowest concentration and lowest percentages of D- and L-methamphetamine that HHS-certified laboratories were capable of accurately quantifying. Additionally, the nasal inhaler and standard solutions studies, which were conducted in only four laboratories, revealed that the L-TPC derivatizing reagent was not optically pure. The PT quantification study would help to assess how optical impurities in chiral derivatizing reagents affect the accuracy of enantiomeric determinations.

Tables 2 through 5 (see Appendix 1) show the summarized D-methamphetamine percentage results obtained for the PT samples. Correct results were defined as reported D-methamphetamine percentages within 20% or 2 standard deviations of the group mean. All reported percentage results for a PT sample were used to determine the % D-methamphetamine group mean. Some laboratories did not report a percentage when the D-methamphetamine concentration was less than the laboratory's limit of quantitation (LOQ); these results were not included in the group mean.

As detailed in the discussion below each table, the results of the PT samples targeted to contain 0% to 20% D-methamphetamine consistently demonstrated D-methamphetamine group means of 1% to 3% above the expected percentage. These results are consistent with the nasal inhaler and standard solution studies, which also demonstrated a high bias that can be attributed to the optical impurity of the L-TPC reagent. Impurities in enantiomer analysis using L-TPC have been reported by others to be 2% to 12% (6, 7).

Another finding of the quantification study was that laboratories using isotopic internal standards to quantify enantiomers obtained D- and L-methamphetamine concentrations consistent with the total methamphetamine concentration determined using the routine amphetamines confirmatory assay. For these laboratories, the total methamphetamine concentrations were within ±20% of sum of the methamphetamine enantiomers for all PT samples. The average difference of the total concentration and enantiomer sum was 5.7%. This finding supports the use of internal standards, which is required in routine confirmatory drug testing, in enantiomeric determinations.

Statistical Analysis

To investigate whether the percentage of D-methamphetamine in a sample affected a laboratory's ability to accurately measure the percentage, the mean absolute value of the difference between the target and reported D-methamphetamine percentages for each PT sample was determined. This showed that the laboratories' ability to measure % D-methamphetamine changed as the percentages changed. The mean absolute value of the differences was largest for the 5% sample (2.85) and smallest for the 20% sample (1.87). The values for the 0% and 10% samples were 2.47 and 2.50, respectively, similar to that of the 5% sample. This suggests that percentages at 10 and below were more difficult to measure accurately.

The next step was a generalized randomized complete block design (GRCBD) analysis using a general linear model procedure in SAS/STAT® software (version 9.2). The overall results for the GRCBD were highly significant (p-value <0.0001), indicating that differences exist in the laboratories' ability to measure different percentages of D-methamphetamine.

Tukey's Least Significant Difference procedure in SAS was used to determine which % D-methamphetamine levels were statistically different. The mean absolute value of the difference for the 20% sample was significantly lower than the mean absolute value for the other three samples (0%, 5%, and 10%). No other significant differences were found. This means that the laboratories obtained more accurate results at the 20% level, and less accurate results at the lower percentage levels.

Summary

The statistical analysis of the PT sample data supports the current guidance for interpreting positive methamphetamine drug test results: >20% D-methamphetamine indicates a source other than an OTC nasal inhaler. The PT results, obtained using current laboratory testing methods for federally regulated specimens, do not support a change in guidance at this time. This conclusion is based on the D-methamphetamine group mean percentages for the OTC nasal inhalers, the standard solutions, and the PT samples, which revealed biases due to impurities in the L-TPC chiral derivatizing reagent used by 23 of the 24 HHS-certified laboratories that perform methamphetamine enantiomer analysis.

There are several options that would provide a basis for lowering the current 20% D-methamphetamine limit for interpreting methamphetamine enantiomer results from GC/MS assays using chiral derivatization. The first and simplest option would be for laboratories to adjust the percentage obtained for D-methamphetamine to account for the amount of impurity in the chiral reagent used for derivatization. As shown in these studies, results obtained with the MTPA reagent would require no correction, since there was no evidence of impurities. A second option would be for laboratories to use a chiral reagent that contained impurities of less than 1%. It is suggested that a chiral reagent other than MTPA be investigated because the reagent is corrosive and continued use can damage equipment. Another option would be the use of chiral columns. Since the completion of these studies, one certified laboratory has implemented a methamphetamine enantiomer LC/MS/MS assay using a chiral column. The laboratory validated the assay in accordance with program requirements and submitted data demonstrating acceptable performance.

Recommendations to Enhance D- and L-Methamphetamine Analysis

It is suggested that laboratories enhance their enantiomer assays by implementing procedures similar to those listed below.

1. **Verify the chiral purity of derivatizing reagent using samples containing L-methamphetamine only and D-methamphetamine only.**

   Prior to placing a new L-TPC lot into service, laboratories should verify that the reagent contains no more than 3% impurity (i.e., the combined impurities of the standard and the derivatizing reagent are not more than 3%). Note: While one may not be able to determine the purity of the derivatizing reagent directly, the total amount of chiral impurities from the reagent plus the standard compound can be used to test new reagent lots.

2. **Verify each lot of quality control material (stock standards and QC samples) for enantiomeric purity. The D-methamphetamine and L-methamphetamine used in these materials should be at least 99% pure.**

   A supplier's certificate of analysis stating the purity and chemical identity of standards is not sufficient. The laboratory should ensure that chiral impurity of the standard is no more than 1% (i.e., the combined impurities of the standard and the derivatizing reagent are not more than 3%) prior to placing the standard into service. See note above in number 1.

3. **Use stable isotope internal standards and quantify D- and L-methamphetamine.**

   Quantification using a stable isotopic internal standard allows laboratories to determine enantiomer percentages based on concentrations. The laboratory can determine the assay upper limit of linearity for each enantiomer, which is not possible when using only peak areas to determine D- and L-methamphetamine percentages.

4. **Include appropriate calibrators and controls in each batch:**

   - A calibrator containing L-methamphetamine at the cutoff concentration and D-methamphetamine at the cutoff concentration.
   - An L-methamphetamine control (containing a drug from a separate source than the calibrator) at a concentration sufficient to demonstrate the amount of D-methamphetamine due to impurities.

     This control will demonstrate any contribution to D-methamphetamine concentrations due to impurities in the standards and/or chiral derivatizing reagent.
   - A negative control (no D- or L-methamphetamine).
   - A control targeted to contain total methamphetamine at the cutoff concentration (250 ng/mL), with D-methamphetamine at the percentage used to interpret results (50 ng/mL D-methamphetamine and 200 ng/mL L-methamphetamine).

     This control will demonstrate that the assay is sufficiently sensitive.

5. **Compare the sum of the D- and L-methamphetamine concentrations from the enantiomer assay to the methamphetamine concentration from the amphetamines confirmatory assay.**

   This will document the consistency of the laboratory's analytical data for a specimen.

References

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Francis Esposito, Ph.D., DABFT, has over 40 years of toxicology experience including drugs of abuse testing of urine, oral fluid, and blood. He has worked as a Senior Research Forensic Scientist in the Center for Forensic Sciences (CFS) at RTI International since 2002 and currently serves as a Co-Deputy Director for the National Laboratory Certification Program (NLCP), under contract with the Department of Health and Human Services (HHS). In addition to managing the Performance Testing (PT) activities of the NLCP, Dr. Esposito manages RTI's Oral Fluid Proficiency Testing program and commercial PT products. Dr. Esposito is a diplomate of the American Board of Forensic Toxicology (ABFT). He is a former Responsible Person of two HHS-certified laboratories and is an inspector for the NLCP and ABFT.

Appendix 1

Table 2. 20% D-methamphetamine PT Sample Results

	PT #15	PT #12	PT #9	PT #4
D-MAMP (target ng/mL)	1000	200	100	50
L-MAMP (target ng/mL)	4000	800	400	200
% D-MAMP Mean*	21.5%	21.1%	21.1%	21.7%
Reported Range	18%–29.7%	18%–26%	17%–27%	19%–28%
Correct Results	23	23	23	22
20%/2 SD Errors	1	1	1	1
Labs Reporting <20%	0	0	0	1

** % D-methamphetamine group mean includes all reported percentages.*

Table 2 Discussion: Four PT samples (#15, #12, #9, and #4) contained various amounts of D- and L-methamphetamine, with a target ratio of 20% D-methamphetamine. The % D-methamphetamine group means were very similar and were 1.1% to 1.7% higher than the target, including the lowest D-methamphetamine target concentration of 50 ng/mL in PT sample #4. The ranges of reported results for each PT sample were also similar, but with a high bias of the range to the target percentage. One D-methamphetamine percentage was not within ±20% or 2 SD of the group mean for each PT sample, and one laboratory reported the percentage as "<20%" for PT sample #4 because the D-methamphetamine concentration was less than the laboratory's LOQ.

Table 3. 10% D-methamphetamine PT Sample Results

	PT #14	PT #11	PT #8	PT #3
D-MAMP (target ng/mL]	500	100	50	25
L-MAMP (target ng/mL]	4500	900	450	225
% D-MAMP Mean*	12.3%	12.1%	12.6%	13.0%
Reported Range	10%–19.7%	9%–18%	10%–18%	10%–19%
Correct Results	20	20	20	18
20%/2 SD Errors	2	2	2	1
Labs Reporting <20%	2	2	2	5

** % D-methamphetamine group mean includes all reported percentages.*

Table 3 Discussion: Four PT samples (#14, #11, #8, and #3) contained various amounts of D- and L-methamphetamine, with a target ratio of 10% D-methamphetamine. The % D-methamphetamine group means were similar and were 2.1% to 3% above the target. As with the 20% D-methamphetamine samples, the ranges of reported results for each PT sample were nearly the same, but had a high bias. PT sample #8 had a target concentration of 50 ng/mL of D-methamphetamine, which was the stated LOQ of 11 participating laboratories. Twenty of the 24 laboratories reported correct results for this sample. PT sample #3 had the lowest D-methamphetamine target concentration, 25 ng/mL, which was also the lowest LOQ value, as reported by three participating laboratories. Eighteen of the 24 laboratories reported correct results for this sample.

Table 4. 5% D-methamphetamine PT Sample Results

	PT #13	PT #10	PT #7
D-MAMP (target ng/mL]	250	50	25
L-MAMP (target ng/mL]	4750	950	475
% D-MAMP Mean*	7.7%	7.5%	8.4%
Reported Range	5%–13.1%	5%–13%	6%–14%
Correct Results	20	20	18
20%/2 SD Errors	2	2	0
Labs Reporting <20%	2	2	6

** % D-methamphetamine group mean includes all reported percentages.*

Table 4 Discussion: Three PT samples (#13, #10, and #7) contained various amounts of D- and L-methamphetamine, with a target ratio of 5% D-methamphetamine. Again, the % D-methamphetamine group means were 2.5% to 3.4% above the target with high bias ranges. As with the 10% D-methamphetamine samples, 20 of 24 laboratories reported correct results for the 50 ng/mL D-methamphetamine (PT #10) and only 18 laboratories reported correct results for the 25 ng/mL D-methamphetamine sample (PT #7).

Table 5. 0% D-Methamphetamine PT Sample Results

	PT #17	PT #16	PT #5	PT #18
D-MAMP (target ng/mL]	0	0	0	0
L-MAMP target ng/mL]	4000	1000	500	0
L-AMP (target ng/mL]	150	150	0	0
% D-MAMP Mean*	2.5%	2.3%	2.6%	0.3%
Reported Range	0%–8.8%	0%–9%	0%–9%	0%–4.9%
Labs Reporting ≥5%	3	3	4	0

** % D-methamphetamine group mean includes all reported percentages.*

Table 5 Discussion: Four PT samples (#17, #16, #5, and #18) were prepared with L-methamphetamine of 0 to 4000 ng/mL and no D-methamphetamine. Of these, the three PT samples containing L-methamphetamine had % D-methamphetamine group means of 2.3% to 2.6%. The reported ranges were 0% to 9%. For PT samples #16 and #17, three laboratories reported D-methamphetamine ≥5% and, for sample #5, four laboratories reported D-methamphetamine percentages ≥5%. PT #18, which contained no methamphetamine, had a negligible mean percentage of 0.3% D-methamphetamine because one laboratory reported 4.9% D-methamphetamine.