National Laboratory Certification Program

DRUG TESTING MATTERS

January 2013

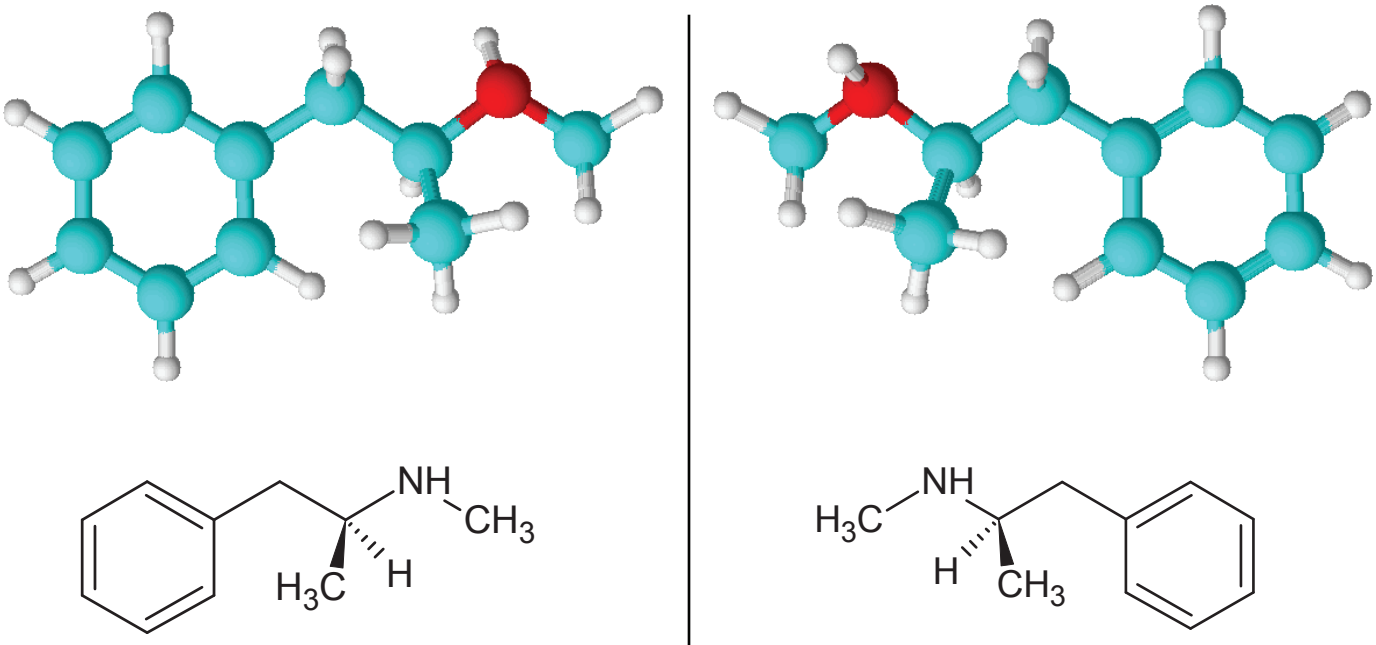
Methamphetamine Enantiomers: Analysis and Result Interpretation

This is the first of a two-part Drug Testing Matters series. This part contains a brief description of methamphetamine stereochemistry and enantiomer analysis, background information on Medical Review Officer (MRO) interpretation of methamphetamine drug test results; and a study summary of methamphetamine purity in over-the-counter (OTC) nasal inhalers and commercial standard materials. The next part will include a study summary of current laboratory testing capabilities and recommendations for methamphetamine enantiomer testing in federally regulated workplace programs.

Methamphetamine Stereochemistry

Isomers are compounds with the same number and kind of atoms (molecular formula), but different structural arrangements of atoms. *Stereoisomers* are compounds with the same bond structure, but the positions of atoms and functional groups differ. These include *enantiomers* which are non-superimposable mirror images and *diastereomers* which are not mirror images. Enantiomers have the same physical properties, but they differ in the way they rotate a beam of polarized light.

Methamphetamine exists as two optically active compounds that are enantiomers. The form of methamphetamine that rotates polarized light to the right is termed dextrorotatory (D-methamphetamine or (S)-(+)-methamphetamine), and the form that rotates polarized light to the left is termed levorotatory (L-methamphetamine or (R)-(-)-methamphetamine).



D-methamphetamine

L-methamphetamine

Figure 1. Methamphetamine Enantiomers

These two forms of methamphetamine have different pharmacological properties. D-methamphetamine is a strong central nervous system (CNS) stimulant used therapeutically or illicitly as a drug of abuse. L-methamphetamine is a weak CNS stimulant with greater vasoconstrictive properties, and is used primarily as a nasal decongestant in non-prescription inhalers (e.g., Vicks® VapoInhaler®). It is also a metabolite of selegiline, a prescription medication used in the treatment of Parkinson's disease.

Methamphetamine Enantiomer Analysis

Amphetamines initial test methods (immunoassays) do not distinguish enantiomers and are sufficient only to distinguish negative specimens from those that may be positive. The immunoassays have been developed to be more sensitive to D-methamphetamine than to L-methamphetamine, but the cross-reactivities vary for different manufacturers' reagents.

Routine gas chromatography/mass spectrometry (GC/MS) confirmation procedures for methamphetamine are not capable of separating the methamphetamine enantiomers. However, enantiomeric analysis can be accomplished using GC/MS or another technology such as liquid chromatography/mass spectrometry (LC/MS) through the use of a chiral derivatizing reagent or an optically active chiral column. Chiral columns have a chiral stationary phase with which enantiomers interact differently, resulting in different retention times. Chiral derivatizing reagents react with the enantiomers and convert them to diastereomers that are chromatographically separated.

In 2011, 24 of 37 laboratories certified by the Department of Health and Human Services (HHS) performed methamphetamine enantiomer testing (three laboratories also performed amphetamine enantiomer testing). All laboratories used a chiral derivatizing reagent: 23 laboratories used N-trifluoroacetyl-L-prolyl chloride (L-TPC) and one laboratory used (R)-(-) methoxytrifluoromethylphenylacetic acid (MTPA). Both L-TPC and MTPA reagents target the amine group on the enantiomers and convert them to diastereomeric amide derivatives which can be separated on non-chiral columns.

N-[N-[(Trifluoroacetyl)prolyl] methamphetamine

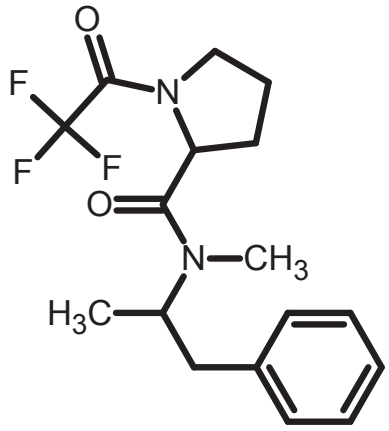


Figure 2. L-TPC Methamphetamine Derivative

N-[Alpha-methoxy-alpha-(trifluoromethyl) phenylacetyl] methamphetamine

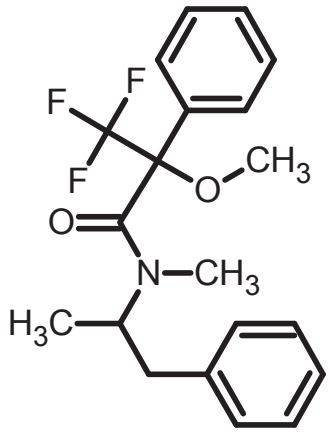


Figure 3. MTPA Methamphetamine Derivative

The optical purity of the chiral derivatizing reagent is important for successful enantiomer analysis. The use of an optically impure derivatizing reagent leads to an optically impure reaction product – resulting in both enantiomers even when the sample analyzed contains only one enantiomer. The derivatizing reagent L-TPC is less optically pure than MTPA. This can be explained by the keto-enol tautomerization of the α-proton on the chiral carbon atom with the neighboring carbonyl group. Because MTPA does not have an α-proton on the chiral carbon atom, this interaction does not occur and the reagent maintains its optical purity (1).

Methamphetamine Result Interpretation

It is the responsibility of the Medical Review Officer (MRO) to interpret a positive methamphetamine drug test result in urine drug testing conducted in accordance with the HHS Mandatory Guidelines for Federal Workplace Drug Testing Programs (2). If a donor has no medical explanation for a positive methamphetamine result and claims to have used an over-the-counter (OTC) nasal inhaler, the MRO can order methamphetamine enantiomer testing to delineate the enantiomeric composition.

The HHS MRO Manual (3) includes guidance for interpreting methamphetamine enantiomer test results as follows. If there is >80% L-methamphetamine, the results are consistent with OTC inhaler use and the MRO verifies the test result as negative for methamphetamine. If there is >20% D-methamphetamine present, the results indicate a source other than an OTC product, and the MRO verifies the test result as positive for methamphetamine. These percentages, provided to HHS-certified laboratories in a 1991 HHS Technical Advisory, are based on laboratory testing capabilities in the 1980s.

Revisions to the HHS Mandatory Guidelines included lowering the methamphetamine confirmatory test cutoff from 500 to 250 ng/mL. After the lower cutoff was implemented (effective October 1, 2010), some MROs reported an increase in the number of methamphetamine positive results with percentages of D-methamphetamine <20%. In some of these cases, the total methamphetamine concentration was >5,000 ng/mL, with L-methamphetamine well above the 1,400 ng/mL maximum reported from typical nasal inhaler use (4). Additionally, there have been reports from the Drug Enforcement Administration of clandestine methamphetamine containing low amounts of D-methamphetamine following federal steps to reduce the availability of ephedrine and pseudoephedrine often used in the production of illicit methamphetamine.

These factors prompted the Substance Abuse and Mental Health Services Administration (SAMHSA) to direct studies by RTI International (RTI) to evaluate the testing capabilities of HHS-certified laboratories and to reassess the current HHS interpretation guidance (5). We designed studies to (1) determine the amount of methamphetamine enantiomers in OTC nasal inhalers, (2) determine the purity of commercial methamphetamine enantiomer standards, and (3) prepare and evaluate results of a methamphetamine enantiomer proficiency testing (PT) set to determine the lowest concentration and lowest percentage of D-methamphetamine that HHS-certified laboratories could accurately quantify. The results of the PT set will be discussed in the next issue of Drug Testing Matters.

OTC Inhaler Study

Six OTC nasal decongestant inhalers were purchased from various local pharmacies: three Vicks® VapoInhalers® with different lot numbers and three pharmacy store brand inhalers (i.e., Walgreens, CVS and Kerr). The plastic cylinder of each inhaler was sliced open and the swab containing the drug was removed, immersed in 10 mL of methanol in separate, capped scintillation vials and mechanically rocked for one hour. The methanolic solutions were diluted with deionized water and sent to two HHS-certified laboratories (designated Laboratory A and Laboratory B) for methamphetamine enantiomer testing. Laboratory A used the L-TPC chiral derivatizing reagent and Laboratory B used the MTPA derivatizing reagent. Laboratory A also analyzed each solution for D- and L-amphetamine.

Laboratory A reported D-methamphetamine percentages of 2% to 2.5% for all six inhalers. Laboratory B reported 0% D-methamphetamine for each of the six inhalers. A technical representative from Proctor and Gamble verified by telephone that the percentage of D-methamphetamine in the Vicks® VapoInhaler® is <1%. Laboratory A also analyzed the six inhalers for D- and L-amphetamine and none was found.

Methamphetamine Enantiomer Standard Study

Two D-methamphetamine and two L-methamphetamine drug standards were obtained from different chemical suppliers. Four solutions were prepared and tested by four HHS-certified laboratories. Average D-methamphetamine percentages for the standard solutions are presented in Table I. Each laboratory obtained similar results for the standard solutions from both suppliers.

Table 1. % D-Methamphetamine Results – Standard Solutions

Laboratory	Chiral Derivative	D-Methamphetamine Solution	L-Methamphetamine Solution
A	L-TPC	95.5%	3.5%
C	L-TPC	97.2%	2.3%
D	L-TPC	97.7%	2.3%
B	MTPA	100%	0%

The three laboratories using the L-TPC derivative (Laboratories A, C, and D) found between 95.5% and 97.7% D-methamphetamine in the D-methamphetamine solutions, and between 2.3% and 3.5% D-methamphetamine in the L-methamphetamine solutions. However, the laboratory using the MTPA derivative (Laboratory B) found the D-methamphetamine solutions and the L-methamphetamine solutions to be 100% pure.

Summary

Results from the laboratory using the MTPA reagent agreed with the manufacturers' stated content of the nasal inhalers and the manufacturers' stated purity of D- and L-methamphetamine in the standard materials. However, results from laboratories using the L-TPC reagent were not consistent with the stated content. D-methamphetamine was reported as 2% to 2.5% of total methamphetamine in the nasal inhalers, as 95.5% to 97.7% of total methamphetamine in the D-methamphetamine solutions, and as 2.3% to 3.5% of total methamphetamine in the L-methamphetamine solutions. Although limited to four laboratories, the results of the inhaler and standard solution studies demonstrate that the L-TPC chiral reagent is not as optically pure as the MTPA reagent.

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.