Using Amphetamine Isomer Ratios to Determine the Compliance of Amphetamine Abusers Prescribed Dextedrine

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Abstract

The amphetamine isomer ratios (l-amphetamine/d-amphetamine) in 373 urine specimens submitted for analysis over a two-year period have been determined using a chiral derivatizing agent in conjunction with a gas chromatograph fitted with a nitrogen-specific detector. All of the specimens were collected from known or suspected amphetamine abusers, some of which were prescribed dextedrine for maintenance and detoxification. The mean (± 1 standard deviation [SD]) l/d-amphetamine isomer ratio for 147 specimens from compliant subjects prescribed dextedrine was 15.0% (± 4.9%). The mean (± 1 SD) l/d-amphetamine isomer ratio from 165 subjects abusing illicit amphetamine was 98.5 (± 27.5%). The calculation of l/d-amphetamine isomer ratios in urine has been found to be a rapid method for determining the compliance of subjects prescribed dextedrine and is therefore a useful technique for the continued management of amphetamine abusers.

In addition, 17 specimens of illicit amphetamine powder (assumed to be a racemic mixture) were submitted to the laboratory for analysis. Using a combination of gas chromatography with and without chiral derivatization, the powders were found to have a mean l/d-amphetamine isomer ratio of 89.2% (range 72.2% to 98.3%) and mean purity (w/w) of 21.5% (range 3.4% to 71.0%) relative to pure dl-amphetamine substance.

Introduction

Amphetamine was first commercially available in 1932 as a nasal mucosa dilator and was used extensively during World War II to combat fatigue (1). However, it is the euphoric and stimulant properties that have resulted in amphetamine becoming Britain's most commonly abused stimulant, second only to cannabis in the overall league table of illicit drug use (2). The number of persons found guilty, cautioned, or dealt with for drug offenses relating to amphetamine in the U.K. increased from 3532 in 1991 to 10,364 in 1995, with the number of seizures rising from 6821 to 15,443 over the same period. The quantity of amphetamine seized also increased from 421 kg in 1991 to 819 kg in 1995 (3).

Amphetamine is one of the most potent sympathomimetic amines in stimulating the central nervous system with dose-related psychic effects (4). It can be abused by oral, nasal, or intravenous routes. The development of tolerance, combined with the desire for the feeling of power, hyperactivity, and euphoria, can lead a heavy intravenous user to inject 5–8 g per day. It should be noted that abused street amphetamine, also known as whizz, sulphate, or speed, is of relatively low purity, typically around 5% (1,2).

Illicit amphetamine is produced as a racemate composed of two isomers, the d-isomer and the l-isomer, in a ratio of 1:1. Dextedrine, the d-isomer of amphetamine, has 3–4 times the central stimulant activity of the l-isomer and is used as an effective substitute and harm-reduction mechanism for subjects undergoing amphetamine stabilization and withdrawal-treatment programs. The l-isomer has been found to be slightly more potent in its cardiovascular effects, leading to increased blood pressure and arrhythmias at high doses (4,5).

The renal elimination of amphetamine, a weak base, is pH dependent with an elimination half-life ranging between 7 and 34 h. Normally, around 30% of the dose is excreted unchanged in the urine, but this may increase to around 60–70% under acidic conditions (elimination half-life of 8 to 10.5 h) or decrease to between 1 and 7% (elimination half-life of 16 to 31 h) in alkaline urine specimens (1,6).

The urinary elimination of amphetamine has been found to be stereospecific with the elimination half-life of the d-isomer (average of 13 h) being slightly shorter than the l-isomer (average of 17 h) under alkaline conditions (1,7). Under acidic conditions, the clearance through renal excretion is more rapid, so the difference in isomer half-life is minimized (1). All of these drug-clearance issues will impact the detection of amphetamine in the urine of drug abusers and the subsequent interpretation of analytical findings.

As with the use of methadone as a detoxification treatment for opiate abuse, it is necessary to monitor for compliance in
subjects prescribed dexedrine. This is performed to ensure that the treatment regime is being adhered to, that street amphetamine is no longer being abused, and that leakage of pharmaceutical-grade/purity amphetamine onto the illicit markets from the clinics is not occurring. This audit of the treatment can only be achieved through the use of a stereoisomer specific or chiral analytical technique. This is because routine screening techniques using either immunoassay or gas chromatography (GC) are unable to differentiate between the two isomers of amphetamine. Classical analytical techniques can therefore only confirm the presence of amphetamine in urine specimens without determining the relative proportion of its two isomers.

It should be noted that the l/d ratio of pure dexedrine is around 5% because of residual impurities from manufacture (7) and that the l/d ratio of illicit amphetamine should be around 100% because it is produced illegally in a racemic form. This hypothesis was tested by the analysis of 17 specimens of illicit amphetamine powder that were submitted to the laboratory for analysis. These powders were sent to the laboratory by the key drug workers responsible for the care of several subjects known to abuse amphetamine after the users questioned their content and purity following adverse reactions subsequent to their use. The submissions were accompanied by claims of the powders being “too hot to handle” and “having to use heroin to come down”. The powders were therefore analyzed for clinical rather than judicial purposes.

Methods

Materials and equipment

Pure amphetamine substances (d-, l-, and dl-amphetamine), prazepam, and N-trifluoroacetyl-l-prolyl chloride were purchased from Sigma (Poole, Dorset, U.K.). Sodium hydroxide pellets were AnalR grade obtained from BDH (Lutterworth, Leicestershire, U.K.). The 12-mL polypropylene test tubes and the 1.9-mL Eppendorf tubes were from Sarstedt (Leicester, Leicestershire, U.K.). Butyl acetate was HPLC grade from Sigma-Aldrich (Gillingham, Dorset, U.K.). The GC system consisted of a Pye Unicam PU4550 GC with a PU4700 Autojector (Pye Unicain Ltd., Cambridge, U.K.) fitted with an AI nitrogen-specific detector (AI Ltd., Cambridge, U.K.) linked to a Hewlett-Packard HP3394 integrator (Stockport, Cheshire, U.K.). The analytical capillary column used was a 30-m DB17 column (0.53-mm internal diameter) with a 50% phenyl-methyl-polysiloxane stationary phase (1-μm film thickness) purchased from Jones Chromatography (Hengoed, Mid Glamorgan, U.K.).

Urine analysis

Random spot urine specimens obtained from subjects thought to be abusing amphetamine or prescribed dexedrine were collected into plain, sterile 25-mL universal plastic containers without preservative. The specimens were stored at −20°C prior to analysis.

Urinary measurements of the isomers of amphetamine were performed by capillary GC with nitrogen-specific detection. The column over temperature was 220°C with the injector and detector set at 260°C. The carrier gas was helium at a head pressure of 8.5 psi with nitrogen used as the make-up gas for the detector.

The extraction was performed in a capped 1.9-mL polypropylene Eppendorf tube. Into the Eppendorf tube were placed 100 μL of 5M sodium hydroxide, 700 μL of urine specimen, and 250 μL of butyl acetate. The tube was capped and vortex mixed for 30 s, then centrifuged at 13,000 rpm for 10 min. A 150-μL aliquot of the supernatant organic phase was then transferred by pipette into a second Eppendorf tube where 5 μL of the chiral derivatizing agent N-trifluoroacetyl-l-prolyl chloride was added. The tube was capped, vortex mixed for 10 s, and incubated at room temperature for 15 min to allow the derivatization reaction to occur. After the incubation period, the reaction was stopped by the addition of 1.0 mL 0.01M sodium hydroxide. Again the tube was capped and vortex mixed for 30 s, then centrifuged at 13,000 rpm for 10 min. A final aliquot (100 μL) of the supernatant organic layer was transferred into a 12-mL polypropylene tube and evaporated to dryness at 45°C. The
residue was reconstituted in 100 μL of butyl acetate and transferred to an autosampler vial, and 1.0 μL was injected onto the column.

Under these conditions, the retention times for the \( l \)-isomer of amphetamine was 5.69 min and that for the \( d \)-isomer was 6.06 min with baseline separation being achieved. The total run time was 10 min per specimen (Figure 1). The amphetamine derivatives were well resolved, and no interferences were noted from other drugs, including phentermine, methamphetamine, methylenedioxymethamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxymethylamphetamine (MDEA), phenylpropanolamine, ephedrine, or with diethylpropion (the common sympathomimetic amines detected in the abuse situation in the U.K.) postderivatization. No other interferences with the performance of the assay as a result of specimen analysis have been observed over the past three years that the method has been in routine use.

Results interpretation

The pragmatic 20% cutoff for the \( l/d \) ratio for compliance that is applied throughout this study, was derived from the analysis of urine specimens obtained from 25 subjects prescribed dexedrine and known by their consultant psychiatrist to be stable and compliant. The mean \( l/d \)-isomer ratio for these subjects was found to be 7.5%; with a maximum \( l/d \)-isomer ratio of 16%. It should be remembered that pure dexedrine yields an \( l/d \)-isomer ratio of around 5% (7). An additional allowance was then made for possible differences in the isomer elimination half-life because \( d \)-amphetamine is cleared more rapidly than the \( l \)-isomer in alkaline urine conditions, although as stated previously, this should not be a problem in acidic urine specimens.

Illicit powder analysis

Illicit amphetamine powder was dissolved in ultrapure water to produce an initial solution at a concentration of 1.0 mg/mL. This was then further diluted to give a 10 mg/L aqueous solution, which was then extracted as follows: the extraction was performed in a 1.9-mL polypropylene Eppendorf capped tube. Into the Eppendorf tube were placed 100 μL of 5M sodium hydroxide, 700 μL of the 10 mg/L aqueous powder solution, and 150 μL of internal standard solution (prazepam at a concentration of 10 mg/L in butyl acetate). The tube was capped and vortex mixed for 30 s, then centrifuged at 13,000 rpm for 10 min. A 100-μL aliquot of the organic supernatant phase was transferred to an autosampler vial, and 1.0 μL was injected onto the column. The peak height of the amphetamine peak obtained for the powder was then compared against the peak height obtained for a 1.0 mg/L pure amphetamine standard extracted by the same technique. The percentage purity was then calculated on a weight-to-weight basis taking into consideration the differences in the concentrations of the solution and the standard.

Results

The chiral amphetamine assay described was found to be linear over the concentration range of 0–50 mg/L (n = 6), with the linear regression coefficients being calculated as 0.9975 for \( l \)-amphetamine and 0.9971 for \( d \)-amphetamine. The within-batch variations for six replicates at a concentration of 12.5 mg/L were calculated to be 3.1% for the \( l \)-isomer of amphetamine and 3.7% for the \( d \)-isomer. This illustrates the potential for a quantitative assay should it be required. No internal standard was used for this work because the proposed application was to determine the isomeric ratios of \( d \)-amphetamine compared to \( l \)-amphetamine. However, the linear regression coefficients and the within-batch variation data illustrate the robustness of the assay.

A total of 373 urine specimens obtained from drug-dependency units, community health teams, and general practitioners have been analyzed by this method over the past three years. The \( l/d \)-amphetamine ratios were calculated as the peak height of the \( l \)-isomer divided by the peak height of the \( d \)-isomer and expressing the value as a percentage. The derived \( l/d \)-amphetamine ratios have allowed the discrimination of street amphetamine abusers from those compliant on dexedrine. Using the already determined 20% ratio as the upper limit associated with dexedrine compliance (see Methods section), all specimens from subjects who were prescribed dexedrine and had a \( l/d \)-isomer ratio determined to be below 20% (n = 147) were taken to be compliant with their prescribed medication and placed in Group 1 (Figure 2). Those subjects who were prescribed dexedrine but had determined \( l/d \)-isomer ratios between 20% and 50% (n = 61) were assumed to be supplementing their dexedrine with illicit amphetamine and are illustrated as Group 2 in Figure 2.

Those subjects with calculated \( l/d \)-isomer
ratios above 50% \((n = 165)\) were assumed to be taking illicit amphetamine or to have been prescribed dextedrine but also mainly or only using illicit amphetamine. This group is illustrated as Group 3 in Figure 2.

Long-term monitoring of several subjects over the study period to observe the compliance of these subjects has been achieved. Following the reporting of a high \(l/d\)-amphetamine ratio subsequent to chiral analysis of their urine specimens, it can be seen that subjects' deviation from the prescribed dextedrine stabilization and detoxification program is controlled. This is reflected in the \(l/d\)-isomer ratios, which can be seen to fall in those who are compliant. The results obtained for four such subjects are shown in Figure 3.

In addition, any potential intra-individual variation in the elimination of the two isomers of amphetamine that may arise because of individual elimination pharmacokinetics can be studied by long-term monitoring. Subject 5 (Figure 4) shows long-term compliance with \(l/d\)-isomer ratios ranging between 16% and 19% over a period of 11 months. Subject 6 (Figure 4) was studied for a period of 2 months and has \(l/d\)-isomer ratios of 10% to 16%. Subject 7 (Figure 4) was studied for 22 months but was apparently found to deviate from the prescribed dextedrine regimen with \(l/d\)-isomer ratios ranging between 15% and 33%. This would appear to imply that the prescribed doses may have been insufficient to control the subject's drug craving and resulted in occasional supplementation with illicit amphetamine.

The isomer ratios and weight-to-weight purities of the illicit powder specimens submitted for identification purposes are shown in Table I. The appearance of the powders varied from white crystalline material to yellow powder. The purities were calculated on a weight-to-weight basis against an amphetamine standard prepared in-house from pure \(dl\)-amphetamine substance. In all, 17 specimens were analyzed and were found to give a mean \(l/d\)-amphetamine isomer ratio of 89.2% (range 72.2% to 98.3%) and a mean weight-to-weight purity of 21.5% (range 3.3% to 71.0%) relative to the pure amphetamine substance.

**Discussion**

Of the 373 specimens analyzed in this study, 90 (24%) were from subjects prescribed dextedrine, but analysis detected an \(l/d\)-amphetamine isomer ratio greater than 20%. This could possibly indicate that the dextedrine prescribed was insufficient to maintain the subjects emotional condition and drug cravings, or that the prescribed dextedrine was being sold to obtain supplies of illicit amphetamine. One instance of this hypothesis is illustrated in Figure 4 where Subject 7 is seen to be periodically compliant but occasionally to supplement the dextedrine prescription with illicit amphetamine, leading to elevated \(l/d\)-isomer ratios (range 15% to 33%). The isomer ratios obtained for two subjects monitored over a long period, Subjects 5 and 6 (Figure 4) show that intra-individual variation in the handling of amphetamine isomers should not be responsible for such wide variations. In these cases only limited variations in the \(l/d\)-isomer ratios were noted, with the \(l/d\)-isomer ratios for Subject 6 ranging between 10% and 16% over a two-month period and those for Subject 5 only ranging between 16% and 19% over an 11-month period.

The \(l/d\)-isomer ratios obtained for the 147 compliant subjects studied (Figure 2, Group 1) were 15.0% ± 4.9% (mean ± 1 standard deviation), and the ratios for the 165 subjects abusing amphetamine (Figure 2, Group 3) were 98.5% ± 27.5%. These data agree with the work of Tetlow and Merrill (7), who found ratios of 13.2% ± 2.3% for compliant subjects.
Table I. The I/d-Isomer Ratios and Weight-to-Weight Purities of 17 Specimens of Illicit Amphetamine Powder Submitted for Analysis

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<thead>
<tr>
<th>Number</th>
<th>Purity (%)</th>
<th>I/d Ratio (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>6.2</td>
<td>92.9</td>
</tr>
<tr>
<td>2</td>
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<tr>
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<tr>
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<td>72.2</td>
</tr>
<tr>
<td>17</td>
<td>10.0</td>
<td>92.0</td>
</tr>
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</table>

Mean 21.5 89.2
SD 19.4 5.1

and 118.7% ± 19.3% for amphetamine abusers.

The use of dexedrine to act as a harm reduction treatment and means of stabilization and detoxification of subjects addicted to amphetamine is a growing practice (5). Because of the nature of this treatment, that is, the prescription of a high-purity compound (d-amphetamine) with the potential for abuse, it is essential to monitor subject compliance. This monitoring can only be performed accurately by the use of chiral separation of the isomers of amphetamine. This audit of treatment is now performed routinely by our laboratory to ensure the following: 1. That all subjects prescribed dexedrine are compliant during their harm reduction, maintenance, and detoxification programs. 2. That the supplementing of prescribed medication with illicit street amphetamine is not occurring. This would imply that inappropriate dosing protocols were being followed for individual subjects, and patient management problems may arise. 3. That the leakage of prescriptions onto the illicit market is not occurring.

The I/d-amphetamine ratio for the illicit powders studied ranged from 72.2% to 98.3% with a mean value of 89.2%, indicating that illicit production of amphetamine generally results in a racemic compound. The purities of the powders were calculated on a weight to weight basis against pure d/-amphetamine substance. These were found to range widely from 3.3% to 71.0%. The purity of illicit amphetamine in the West Midlands is usually around 3% to 6%, and it was therefore initially thought that the higher purity compounds analyzed were possibly from drug dealers rather than drug users.

A more likely explanation was that the local drug dealers had been arrested and high-purity amphetamine was therefore available on the streets, leading to the adverse reactions described by the local amphetamine abusers. The powders were not screened to determine the presence of other drugs or to discern the profile of the cutting agents and impurities of manufacture present in the powders. This was deemed to be outside the remit of the laboratory, and unnecessary for both the users and drug workers involved and the requirements of this study.

From the results described here, it can be seen that a rapid technique for the analysis of the isomers of amphetamine that does not require the use of mass spectroscopy has been developed. This procedure is now routinely applied to audit the compliance of those prescribed dexedrine for the treatment of amphetamine abuse by this laboratory. The method has also been successfully applied to the study of illicit material.

References


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