

The Evaluation of Amphetamine Isomer Ratios in Hair Samples from Amphetamine Users

Kathryn Higgins, Lolita Tsanaclis and John Sullivan.

TrichoTech, Pentwyn Business Centre, Cardiff, CF23 7HB, UK.

www.tricho-tech.co.uk, E-mail: info@tricho-tech.co.uk

Introduction

Amphetamine is a stimulant drug and is available on prescription in many countries for the treatment of a number of disorders such as narcolepsy or obesity. It is also used illegally for recreational purposes and is currently a Class B controlled drug in the United Kingdom.

Amphetamine is a chiral compound consisting of l- and d- isomers. When synthesised, amphetamine is a racemic mixture consisting of equal parts of each isomer. It is known that the d-isomer is most effective at treating patients symptoms and that the l-isomer contributes to unwanted side effects. For this reason d-amphetamine is isolated or combined with a smaller proportion of l-amphetamine for medicinal use. Illicit "street" amphetamine is generally found as the racemic mixture.

The determination of the enantiomeric composition of amphetamine in urine and blood samples has been used as a valuable tool in interpreting drug testing results for the management of amphetamine users. Testing hair for drugs is now well established as a means of monitoring compliance and assessing drug use or abstinence for an individual over longer time periods; however, published data on the determination of amphetamine ratios in hair samples is scarce. This study examines the pattern of l/d amphetamine ratios in relation to the amphetamine levels detected in hair samples and the declaration of amphetamine based medication by the sample donors. A ratio of up to approximately 20 to 30% l/d amphetamine indicates that the sample donor was taking their prescribed medication only, a ratio of between 30 and 50% suggests that the donor was supplementing their medication with some "street" amphetamine, and a ratio above 50% suggests that the donor was using a quantity of "street" amphetamine. This can be used as an aid to the interpretation of the results from routine analysis of hair samples.

Methods

Hair samples are washed with solvent and submitted to alkaline digestion followed by solvent extraction. After solvent evaporation the residues are reconstituted in phosphate buffer. Sample extracts are screened using an enzyme-linked immunosorbent assay (ELISA). The cut-off level for amphetamine in hair samples is 0.5 ng/mg of hair. Samples above the cut-off are extracted using solid-phase extraction with either HXC or MCX cartridges. The final extracts are evaporated to dryness and derivatised with TFAA (Sigma, Poole, UK) before injecting onto a GC-MS system (Agilent, Berkshire, UK).

The amphetamine isomer ratios (l-amphetamine/d-amphetamine) for 106 samples of hair that tested positive for amphetamine using GC-MS have been determined. These samples are from a very heterogeneous group and reasons for analysis vary (Figure 1). The isomer ratios were determined using a 30-metre chiral column (Restek, Rt-BDEXCst-TM 30m x 0.25mm x 0.25µm) installed in either a GC-MS (Agilent) or ion trap GC-MS/MS (Varian, Walton on Thames, UK) to achieve the separation.

All of the specimens were collected from known or suspected amphetamine users, some of which were prescribed Dexedrine (dexamphetamine sulphate) for maintenance and detoxification. The information given by the sample collector is either based on the declaration of the subject being tested or from knowledge of their medical history. This means that when Dexedrine was not declared, it does not necessarily mean that the subject did not use this medication. Samples were coded according to whether Dexedrine or dexamphetamine sulphate had been declared as medication used by the sample donor (Figure 2).

The analysis of variance (ANOVA) of the data was performed, followed by Tukey's test using SPSS (Statistical Package for the Social Services, version 6.1, Chicago, IL).

Results

Figure 3 shows the structure of amphetamine. Figure 4 shows the GC-MS/MS chiral separation of the l- and d- amphetamine isomers where the level of amphetamine detected in the hair sample was 101.8 ng/mg and the donor had declared dexamphetamine sulphate as medication. The l/d amphetamine ratio is 100% (1:1), indicating that the sample donor was using "street" amphetamine only.

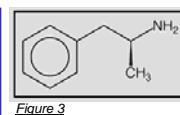
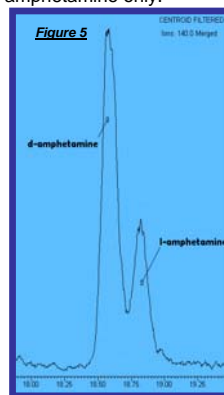
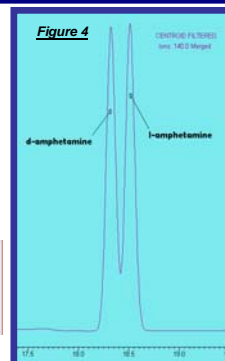
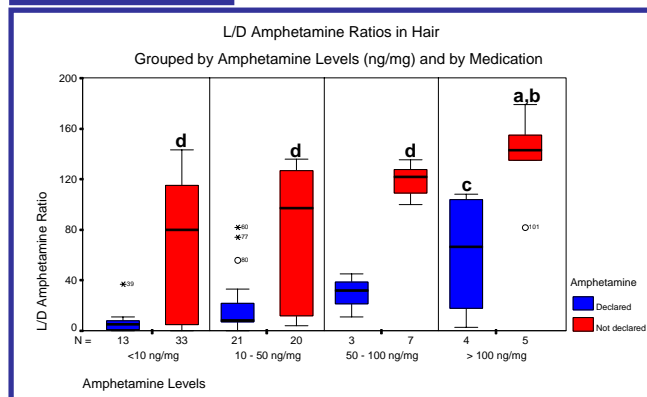


Figure 3

Figure 5 shows the GC-MS/MS chiral separation of the l- and d- amphetamine isomers where the level of amphetamine detected in the hair sample was 57.5 ng/mg and the donor had declared Dexedrine as medication. The l/d amphetamine ratio is 32%, suggesting that the sample donor was supplementing their medication with some "street" amphetamine.



- a: Significantly different from the '<10' and '10 - 50' ng/mg group (medication not declared) (p<0.01)
 b: Significantly different from 'declared amphetamine medication' group at same level (p<0.05)
 c: Significantly different from the '<10' and '10 - 50' ng/mg group (medication declared) (p<0.01)
 d: Significantly different from 'declared amphetamine medication' group up to 100ng/mg (p<0.01)

Figure 6: Representation of L/D amphetamine ratios according to amphetamine levels detected in hair samples and the declaration of medication

Conclusions

- L/D amphetamine ratios were lower in the 41 samples where amphetamine-based medication had been declared, indicating that the subjects were not supplementing with "street" amphetamine as much.
- The results suggest that the subjects using a higher level of amphetamine (levels analysed above 50 ng/mg of hair) and who did not declare amphetamine-based medication, were all using "street" amphetamine.
- The overall pattern of results suggests an increase the supplementing of declared medication with "street" amphetamine in line with increasing levels of amphetamine detected. This may be as a result of increased tolerance to amphetamine and more need to supplement the level of prescribed medication to achieve the desired effects of the drug.
- Hair analysis can be employed as a useful technique in monitoring amphetamine users compliance with medication over a long period of time.

Reasons For Analysis

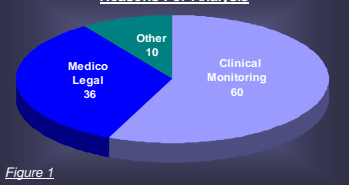


Figure 1

Amphetamine-based Medication

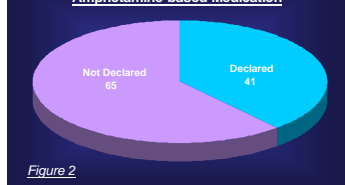


Figure 2