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Some Analytical Data Relevant for the Detection of 1-Benzylpiperazine, a Pharmacological
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Some analytical data relevant for the detection of 1-benzylpiperazine, a pharmacological alternative for amphetamine

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Introduction

1-Aryl-piperazine compounds bind selectively to certain serotonin receptors. Together with their easy availability and their so-called legal status, these compounds are also potential drugs-of-abuse [1]. Because this may have consequences for sport drug testing, we studied some analytical aspects of 1-benzyl-piperazine (Figure 1). This compound is of special interest because it mimics the psychoactive effects of (S)-amphetamine (Figure 1), although at a higher dosage [2,3]. Like for the amphetamine designer drugs [4], athletes may also use this type of drug for recreational reasons. Currently, 1-benzyl-piperazine is available in capsules and can be bought on the Internet as a synthetic stimulant under the name of A2.

![1-benzyl-piperazine and amphetamine](image)

Figure 1 Structural formulas of 1-benzyl-piperazine and amphetamine

This study specifically gives information regarding the implementation of the detection of 1-benzyl-piperazine in some sport drug testing procedures. The procedures studied were an immunoassay for amphetamines, the screening method for volatile basic compounds (simple liquid/liquid extraction at basic pH followed by GC/NPD analysis) as well as the corresponding confirmation method (simple liquid/liquid extraction at basic pH followed by N,O-trifluoroacetylation and GC/MS analysis).
Experimental

Immunoassay for direct analysis

The immunoassay applied was the Amphetamines Abuscreen Online® assay kit (Roche Diagnostic Systems, Somerville, NJ, USA) using the Cobas® Mira Plus system robotic analyser (Roche Diagnostic Systems). The assay was performed according to the manufacturers' recommendations.

Sample preparation for GC/NPD and GC/MS analysis

The liquid/liquid extraction was based on classical screening I procedures [5]. The aqueous phase was made basic with 0.5 mL of 5 M KOH. After adding 3 g of Na₂SO₄, an extraction was performed with 2 mL of tert.-butyl methyl ether in order to isolate the compounds of interest. Diphenylamine was used as an internal standard.

N,O-Trifluoroacetylation was performed by adding 50 µL of trifluoroacetic anhydride and 50 µL of ethyl acetate to a residue containing the compound of interest. The mixture was incubated for 30 min at 65°C. Excess of reagent and organic solvent was removed under a mild stream of nitrogen and the dry residue was re-dissolved in ethyl acetate for GC/MS analysis.

GC/NPD analysis

GC/NPD analysis was performed with a HP GC 5890 series II (Hewlett Packard, Palo Alto, CA, USA) equipped with HP-5MS column (length 14 m, inner diameter 0.25 mm and film thickness 0.25 µm) and a Nitrogen Phosphorous Detector (NPD). The temperature during an analysis run was maintained at 100°C for 1 min, programmed up to 300°C at 15°C/min and maintained there for 5 min. The temperature of the injection port was 250°C. Helium was used as a carrier gas at a flow rate of 1 mL/min.

GC/MS analysis

GC/MS analysis was performed with a HP GC 5890 series II (Hewlett Packard) equipped with HP-5MS column (length 19 m, inner diameter 0.25 mm and film thickness 0.25 µm) and coupled to a HP 5971A Mass Selective Detector (MSD) (Hewlett Packard). The temperature during an analysis run was maintained at 90°C for 1 min, programmed up to 300°C at 15°C/min and maintained there for 5 min. The temperatures of the injection port and transfer line were 270°C and 300°C, respectively. Helium was used as a carrier gas at a flow rate of 1 mL/min. The HP 5971A MSD operates only in the Electron Ionisation mode. Mass spectra were recorded at the standard 70 eV in the range of m/z 40-400.
Results and Discussion

1-Benzyl-piperazine showed some cross reactivity in the Abuscreen Online® Amphetamines immunoassay (Figure 2). The cross reactivity relative to (R,S)-amphetamine was very low (<1%) and was similar to that of the EMIT® d.a.u.® Amphetamines immunoassay [1]. Applying that EMIT® assay already 15 cases with 1-benzyl-piperazine positive urine sample were reported in Swedish Drug-of-Abuse programs [6].

![Graph showing response Abuscreen Online assay for different concentrations of 1-benzyl-piperazine and (R,S)-amphetamine in urine](image)

**Figure 2** Abuscreen Online® Amphetamines response for different concentrations of 1-benzyl-piperazine and (R,S)-amphetamine in urine

1-Benzyl-piperazine proved to be easily detected by the combination of the liquid/liquid extraction and GC/NPD analysis. The relative retention time was 0.839 under the conditions studied. No exact recovery studies (> 80%) nor exact analytical validations were performed, but the limit of detection (LOD) was estimated to be in the range of 250 ng/mL urine. Applying N,O-trifluoroacetylation and GC/MS analysis it showed that the N-trifluoroacetyl (TFA) derivative of 1-benzyl-piperazine had a characteristic mass spectrum (Figure 3). At the LOD level of the GC/NPD analysis it was still possible to confirm the presence of its N-TFA derivative by GC/MS analysis under the conditions described.
Figure 3  Mass spectrum of the N-trifluoroacetyl derivative of l-benzyl-piperazine
Until now no bibliographic data of urinary concentrations of 1-benzyl-piperazine after therapeutic and non-therapeutic dosages have been described in literature. However, in a Swiss case study it was possible to detect 1-benzyl-piperazine in urine by GC/MS analysis until 7 hours after the intake of two tablets of A2 [7]. A tablet of A2 contains approximately 125 mg of 1-benzyl-piperazine dihydrochloride [1]. The suspected cases in the Swedish Drug-of-Abuse programs were associated with the intake of A2 tablets too. Also was it possible in those cases to confirm the presence of the N-TFA derivative of 1-benzyl-piperazine by GC/MS analysis [6].

Conclusions

1. The implementation of 1-benzyl-piperazine in relevant analytical procedures in sport drug testing can easily be achieved in classical screening procedures and if required in some commercial immunological assays for the detection of amphetamines.

2. As a confirmation procedure the use of the N,O-trifluoroacetylation derivatisation step results in a characteristic mass spectrum.

References


