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Some analytical data relevant for the detection of 1-benzyl-piperazine, a pharmacological alternative for amphetamine

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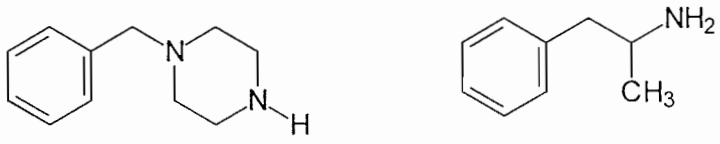
Introduction

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 amphetamine

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 Na_2SO_4, 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°/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 °/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].

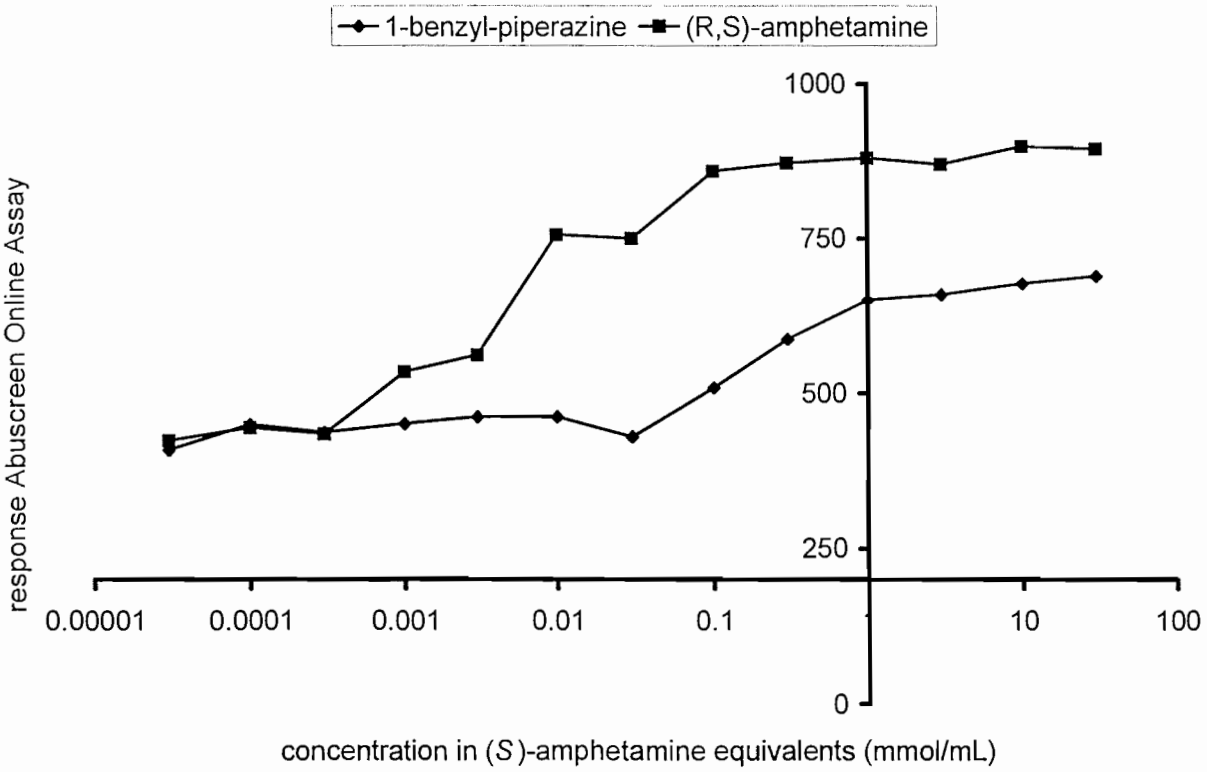


Figure 2 Abuscreen Online® Ampehetamines response for different concentrations of 1-benzyl-piperazine and (*R,S*)-amphetamine in urine

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.

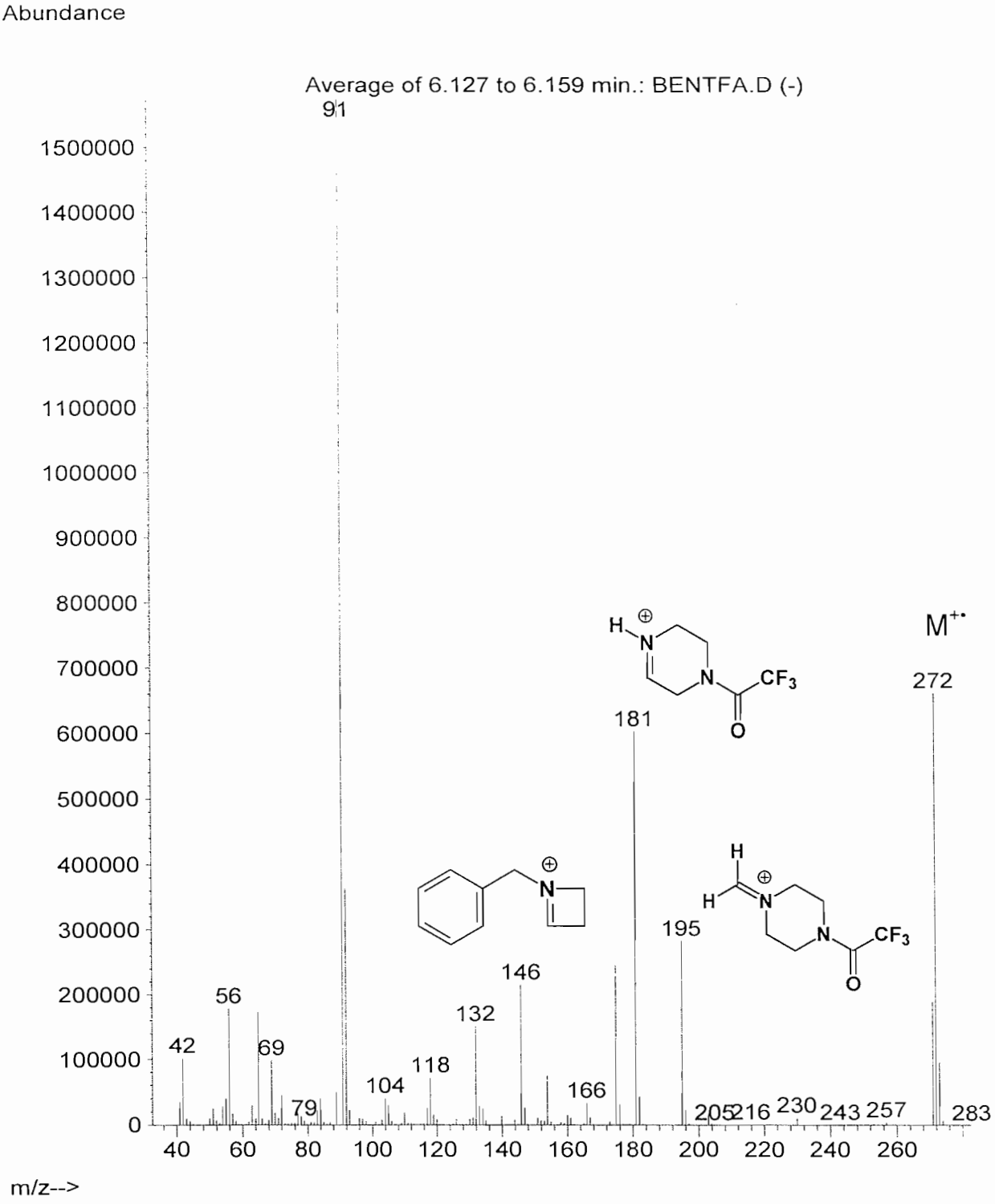


Figure 3 Mass spectrum of the *N*-trifluoroacetyl derivative of 1-benzyl-piperazine

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 I 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

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