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*Technical Note*

# A2 (*N*-Benzylpiperazine) a New Drug of Abuse in Sweden

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## Abstract

**_N_-Benzylpiperazine was tested in the beginning of the 1970s as a possible antidepressant drug. However, in both animal and human studies, it was shown to possess amphetamine-like properties, and any further studies were stopped. In a forensic autopsy case in 1999, we found a substance so far unknown to us in the chromatogram of our method used for amphetamines. We could swiftly identify this compound as _N_-benzylpiperazine because of information given to us by a newly formed network comprising, among others, customs and the police. Since then, we have found _N_-benzylpiperazine in several cases, among them 11 cases from a number of prisons.**

## Introduction

*N*-Benzylpiperazine is a known central nervous system stimulant, with effects comparable to the effects of *S*(+)-amphetamine. *N*-Benzylpiperazine is known as "A2" in Sweden from its alternative chemical name 1-benzyl-1,4-DIAZA-cyclohexane.

It is said to trigger the release of dopamine and norepinephrine and inhibit the uptake of dopamine, norepinephrine, and serotonin.

*N*-Benzylpiperazine was originally synthesized as a potential antihelminthic agent. Later, *N*-benzyl-piperazine-picolinyl fumarate, which has benzylpiperazine as its active metabolite, was introduced, and subsequent studies indicated a potential antidepressant activity (1,2). However, in animal studies, hyperactivity, involuntary head movements, and a reduction of reaction time in shock-avoidance tests were found, indicating that the compound had effects similar to those produced by *S*(+)-amphetamine. Pharmacodynamic studies in humans, in which the effects of *N*-benzylpiperazine and *S*(+)-amphetamine on various aspects of human behavior and autonomic function were compared, reported a close similarity between the two compounds (3,4). Another study made by Campbell et al. (5) compared subjective and autonomic effects of *S*(+)-amphetamine, *N*-benzylpierazine, and a lactose dummy in a group of 18 former amphetamine addicts. In this double-blind study, 100 mg *N*-benzylpiperazine and 10 mg *S*(+)-amphetamine produced indistinguishable subjective effects, and both compounds were liked by the participants. It was concluded that *N*-benzylpiperazine was liable to abuse, and because of that further clinical studies with *N*-benzylpiperazine as a potential antidepressant were

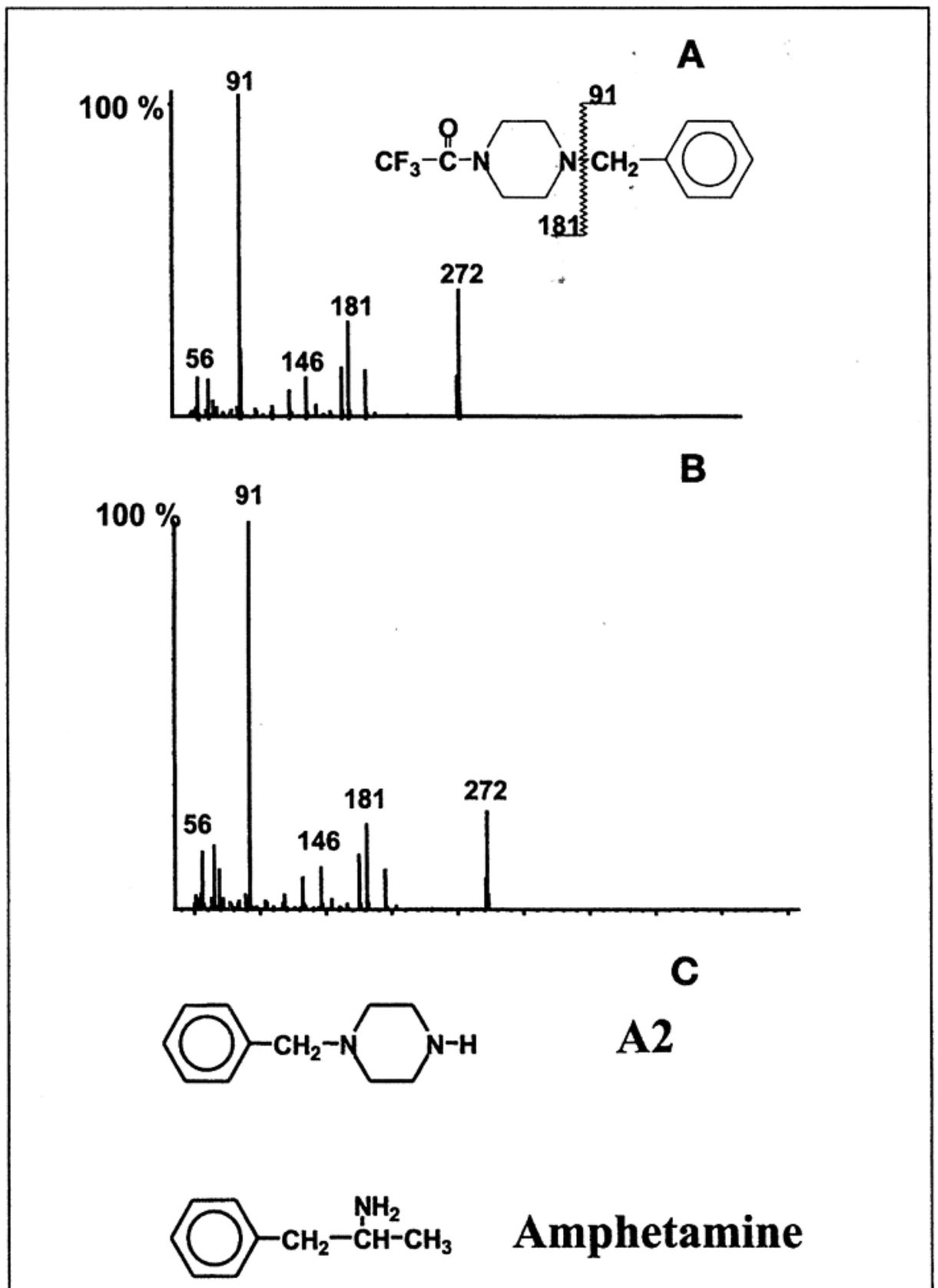


**Figure 1.** Reference spectra of *N*-benzylpiperazine derivatized with trifluoroacetyl (A), mass spectra of the unidentified peak after full-scan analysis (B), and chemical structures of *N*-benzylpiperazine (A2) and amphetamine (C).

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stopped. In view of this work, Campbell et al. (5) suggested that it would be advisable to place A2 under statutory control.

In 2002, *N*-benzylpiperazine was classified as a controlled substance in the United States, and in Sweden it is regulated under the Act on the Prohibition of Certain Goods Dangerous to the Health since March 1, 2003.

It is not classified in Norway, Australia, Canada, or in any other European Union (EU) member state, and, as far as we know, not in any other country than those mentioned so far.

The chemical structure of *N*-benzylpiperazine has several similarities with the structure of amphetamine (Figure 1).

On the Internet, information can be found about the compound, including descriptions of how to synthesize the substance. The drug is taken orally, with doses in the range of 75-250 mg, and the potency is about one-tenth of the potency of *S*(+)-amphetamine.

In September 1999, there was a seizure of an unknown substance in Stockholm, Sweden. The analysis revealed that the substance was *N*-benzylpiperazine.

The Department of Forensic Chemistry is a national laboratory serving forensic medicine, as well as the police and prisons, with analyses of urine and blood samples from suspected users of illicit drugs and pharmaceuticals. When it was known that *N*-benzylpiperazine was on the market in Sweden, we promptly set up a method for the compound. In this article, we describe this method, as well as our experiences from the cases we have analyzed. Part of this paper was presented at the International Society of Forensic Toxicologists meeting in Helsinki, Finland, 2000.

## Methods

All positive results from our screening analysis for amphetamines were confirmed with gas chromatography–mass spectrometry (GC–MS) using a single ion monitoring (SIM) technique. With our confirmation method described here, we usually looked for 10 different amphetamine analogues [amphetamine, metamphetamine, phenmetrazine, ephedrine, norephedrine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), and phentermine].

In urine samples, we quantitated amphetamine and metamphetamine with their own internal standards with the method described. For the rest of the substances, the method was qualitative.

In blood, we quantitated all the analytes mentioned. The ethylacetate extraction gave a much more even recovery than isooctane, and because of that it is possible to quantitate all the substances with acceptable coefficients of variation (CVs). The between-day precision varied between 5.2 and 12.1% for all of the substances, except ephedrine and norephedrine. Their CVs were 22.4–23.8%. For *N*-benzylpiperazine, a linear calibration curve was established between 0.02 and 2.0 µg/g blood, with a CV of 8.5% at 0.05 µg/g and 4.3% at 1.0 µg/g (within day). The recovery was measured to 69.3%.

When we first detected *N*-benzylpiperazine, we saw an unidentified peak in the chromatogram (TIC) of our GC–MS analysis. The peak eluted just before MDMA when derivatized with trifluoroaceticacid-anhydride (TFAA). A full-scan analysis of the blood sample gave the mass spectra shown in Figure 1. The spectra had a molecular ion of *m/z* 272, which would be the molecular ion of *N*-benzylpiperazine as a TFAA derivative. To be sure of the identity, we had to compare the mass spectra and retention time with a reference. *N*-Benzylpiperazine in liquid form (97%) was bought from Chemicon (Acros Organics, Geel, Belgium). The mass spectra of the reference were identical to the mass spectra of the unknown, as can be seen in Figure 1.

### Extraction

*N*-Benzylpiperazine was extracted from blood (1.0 g) with 3 mL ethylacetate after adding 0.5 mL 2M sodium hydroxide (NaOH) and 0.2 µg internal standard (0.2 µg amphetamine-$d_8$ + 0.2 µg metamphetamine-$d_8$ + 0.2 µg MDMA-$d_5$). The organic phase was transferred to a new tube and evaporated under a stream of nitrogen, leaving approximately 50 µL of solvent. After derivatization with 50 µL of TFAA at 60°C for 15 min, the samples were evaporated to dryness and then reconstituted in 40 µL ethylacetate.

In the urine samples, *N*-benzylpiperazine was extracted from the urine (0.2 mL) with 2 mL isooctane after adding 200 µL 2M sodium hydroxide and 0.2 µg internal standard (0.2 µg amphetamine-$d_5$ + 0.2 µg metamphetamine-$d_5$). The organic phase was transferred to a new tube and evaporated under a stream of nitrogen, leaving approximately 50 µL of solvent. After derivatization with 100 µL TFAA at 60°C for 15 min, the samples were evaporated to dryness and then reconstituted in 100 µL isooctane.

### Analysis and identification

GC–MS analysis and electron impact (EI) spectra were performed using an Agilent 6890 GC system interfaced to a 5973 mass selective detector (Agilent, Palo Alto, CA). For the injections, a 7673A autosampler from Agilent was used. The column was a 30-m HP-5 MS (5% phenyl-methylsilicone), with 0.25-mm i.d. and 0.25-µm film thickness (Agilent). Helium was used as carrier gas at a constant flow of approximately 0.9 mL/min. The injections were made in splitless mode with a splitless time of 1.0 min. The injection volume was 1.0 µL.

Full scan spectra were obtained in EI mode, recording from *m/z* 40–550.

After a positive identification of *N*-benzylpiperazine, where a comparison of retention time and full-scan spectra to a standard of *N*-benzylpiperazine was made, the amount of *N*-benzylpiperazine in blood-samples was determined using the area ratios of *m/z* 158 from MDMA-$d_5$-*N*-TFAA and *m/z* 181 from A2-*N*-TFAA, with *m/z* 272 and *m/z* 195 as qualifier ions in SIM analysis.

## Results and Discussion

Since 1999, we have had 56 individual cases in which we have detected *N*-benzylpiperazine. The cases are described in Table I. The cases come from different parts of southern

Sweden, including 11 cases from prisons.

We have had one fatal case, where *N*-benzylpiperazine contributed to the death. This was indeed the first time where we identified A2. The victim was a male born in 1977. He was said to have been taking ecstasy and something called A2. The femoral blood sample taken at the autopsy was screened for amphetamines with a positive result.

In the screening of the blood sample, we used the EMIT®d.a.u.® amphetamines reagent, with polyclonal antibodies. According to de Boer et al. (6), *N*-benzylpiperazine shows a small cross reactivity with the EMIT d.a.u. reagent. We have found that *N*-benzylpiperazine is detected at a level of 15,000 ng/mL of blank urine spiked with *N*-benzylpiperazine (unpublished result). In fact, we have had a positive screening result for amphetamines with the EMIT d.a.u. amphetamines reagent in all of our cases in which we have identified *N*-benzylpiperazine.

Staack et al. (7) describes possible metabolic pathways of *N*-benzylpiperazine in rat and human urine. Especially the metabolites benzylamine and *N*-benzylethylenediamine show structural similarities with amphetamine, and the presence of them in urine may contribute to a positive screening result when screening authentic urine samples for *N*-benzylpiperazine.

The amount of A2 in the autopsy case was measured to 1.7 µg/g blood using the method described. Other findings in this case were MDMA, MDA, and tetrahydrocannabinol (Table I).

The compound seems to have been introduced among drug abusers in Sweden in 1999, and since then several seizures have been made. During 1999, there were 25 seizures. In 2000 and 2001, there were 12 and 13 seizures, respectively. In the period from January 1, 2002, until the beginning of March, 2003, there have been 51 seizures indicating that the drug is increasing on the market.

In a number of cases, we found a mixture of A2 with other substances, mainly amphetamine (Table I). In 21 cases, no other drug than A2 was found. Most of the cases where A2 was found in blood or urine specimens from suspected users are from 2002. It is noted that in the cases found among inmates, A2 was often found as the only drug used.

It is not known with certainty whether the drug users knew that it was a new compound that was taken or whether they thought it was amphetamine. It has been reported from police authorities that users of A2 in prisons have been aware of what they have been using. The reason for using A2 is told to be that *N*-benzylpiperazine has similar effects to amphetamine, but it is not illegal to use and therefore users cannot be prosecuted.

It has been noted that since January 2000, A2 is commercially available on the internet as a so-called synthetic stimulant (6), and the substance qualifies as a new synthetic drug under the terms of the EU joint action on new synthetic drugs (8). However, there have been no reports of any widespread abuse of the substance. This can be due to a lack of proper methods for detecting and identifying the substance. A problem is of course that the substance is not listed in Sweden or in any other country, as far as we know, and that fact can also explain the lack of reports regarding the abuse of A2. There is one case report from Switzerland (9) of fatal brain edema after ingestion of A2 in combination with ecstasy.

In Sweden, the increasing number of seizures and analytical findings justifies the step taken to regulate *N*-benzylpiperazine under the Act on the Prohibition of Certain Goods Dangerous to the Health, as the Swedish National Institute of Public Health has recently done.

**Table I. Description of the Cases, Blood Concentration, and Other Analytical Results**

| Year and Type of Case | Number of Urine Samples | Number of Blood Samples and Concentration* | Other Analytical Results (Number of Cases) |
|---|---|---|---|
| **1999** | | | |
| Drug abusers | 3 | | MDMA, MDA (1) |
| Autopsy | | 1 (1.7) | MDMA, MDA, THC |
| **2000** | | | |
| Inmates | 9 | | THC-COOH (2), amphetamine (1), benzodiazepines (1) |
| Drug abusers | 6 | 2 (1.2; nq†) | THC-COOH (3), amphetamine (3), benzodiazepines (1) |
| Treatment care | 2 | | THC-COOH (2) |
| **2001** | | | |
| Drug abusers | 2 | 1 (nq) | amphetamine (1) benzodiazepines (1) |
| **2002** | | | |
| Inmates | 2 | | amphetamine (2) |
| Drug abusers | 11 | 4 (0.4, 0.4, 0.05, 0.04) | amphetamine (10), THC-COOH (7), benzodiazepines (4), morphine (1) |
| Autopsy | | 1 (1.7) | amphetamine (1), MDMA (1), THC (1) |
| Traffic, drivers | | 3 (0.3, 0.02, 0.02) | amphetamine (2), THC (2), benzodiazepines (1) |
| Treatment care | 2 | | – |
| **2003** | | | |
| Drug abusers | 6 | 1 (0.2) | amphetamine (2), THC-COOH (2), MDMA (1), Kat (1) |
| **Total** | 43 | 13 | |

* Concentration (in parentheses) in µg/g blood.
† nq = Not quantitated.

## References

1. K. Magyar. Pharmacokinetic aspects of the mode of action of EGYPT475; a new antidepressant agent. *Pol. J. Pharmacol. Pharm.* **39:** 107–112 (1987).

2. K. Tekes, L. Tothfalusi, B. Malomvolgi, F. Herman, and K. Magyar. Studies on the biochemical mode of action of EGYPT-47, a new antidepressant. *Pol. J. Pharmacol. Pharm.* **39:** 203–211 (1987).
3. A.D. Munro-Faure, A.W. Peck, C. Pullin, and P.A. Young. Detection of dexamphetamine-like activity in man. A comparison of several tests. *J. Pharm. Chim.* (Paris) **2:** 228–229 (1971).
4. C. Bye, A.D. Munro-Faure, A.W. Peck, and P.A. Young. Comparison of the effects of 1-benzylpiperazine and dexamphetamine on human performance tests. *Eur. J. Clin. Pharmacol.* **6:** 163–169 (1973).
5. H. Campbell, W. Cline, M. Evans, J. Lloyd, and A.W. Peck. Comparison of the effects of dexamphetamine and 1-benzylpiperazine in former addicts. *Eur. J. Clin. Pharmacol.* **6:** 170–176 (1973).
6. D. de Boer, I.J. Bosman, E. Hidvégi, C. Manzoni, A.A. Benkö, L.J.A.L. dos Reys, and R.A.A. Maes. Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic Sci. Int.* **121:** 47–56 (2001).
7. R.F. Staack, G. Fritschi, and H.H. Maurer. Studies on the metabolism and toxicological detection of the new designer drug N-benzylpiperazine in urine using gas chromatography–mass spectrometry. *J. Chromatogr. B* **773:** 35–46 (2002).
8. L.A. King and A.J. Poortman van-der Meer. New synthetic drugs in the European Union. *Sci. Justice* **41:** 200–202 (2001).
9. C. Balmelli, H. Kupferschmidt, K. Reutsch, and M. Schneemann. Fatal brain oedema after ingestion of ecstasy and benzylpiperazine. *Dtsch. Med. Wochensch.* **126:** 809–811 (2000).

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