

A gas chromatography mass-spectrometric method for the quantitative analysis of the recreational drug N-benzylpiperazine in serum

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BACKGROUND

Introduction

A new recreational drug, N-benzylpiperazine (BZP), is available in the UK. It is easily available from internet websites and, despite being entirely synthetic, is frequently marketed as a 'natural' or 'herbal' product, due to its erroneous association with the pepper plant. BZP, whose street names include; A2, Legal X and Frenzy, is just one of the piperazine family of drugs promoted as having similar effects to controlled substances. Although not controlled by the Misuse of Drugs Act, action has been taken against suppliers under the Medicines Act.

BZP is a central nervous system stimulant.¹ It acts on the serotonergic receptors, agonising 5-HT₁ and inhibiting serotonin uptake.² It is also said to trigger the release of dopamine and noradrenaline and inhibit their uptake.^{1,3} BZP was first synthesised in 1944 by the Wellcome Research Laboratories as a potential anti-helminthic agent for livestock.³ Its use for this purpose declined due to poor efficacy and adverse effects such as seizures.³ In human trials BZP resulted in involuntary head movements, reduced reaction times, hyperactivity and agitation.⁴

Studies in the 1970's and 1980's found BZP to possess anti-depressant activity and produce amphetamine-like effects.^{2,4-6} These studies suggested that BZP has approximately one-tenth of the potency of amphetamine (see figure 1 for structural comparison). A double blind study in 1973 even found that former amphetamine addicts were unable to distinguish between equipotent doses of BZP and amphetamine.⁵ A more recent study has shown that combinations of BZP with another piperazine, 1-(3-trifluoromethylphenyl)piperazine (TFMPP) mimics some of the effects of 3,4-methylenedioxyamphetamine (MDMA).⁷

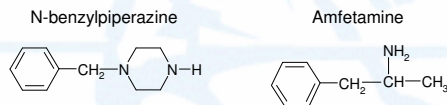


Figure 1. The similarities between the chemical structures of BZP and amphetamine.

Case History

In May 2006, seven patients aged between 18 and 23, presented simultaneously to the Emergency Department (ED) at St Thomas' Hospital, London. All of the patients had been at a single club in the South London area and had ingested tablets, which they believed to be either ecstasy or amphetamine, purchased from the same dealer. The number of tablets ingested per patient ranged from 4 to 9. Two patients had collapsed in the club with witnessed self-terminating grand mal seizures. On arrival in the ED, five of the patients had evidence of a sympathomimetic toxidrome, with dilated pupils, anxiety, agitation and tachycardia. Guy's and St Thomas'

Poisons Unit, London, was contacted for advice on the management of these patients. They were admitted for observation and treated with IV / PO benzodiazepines and IV fluids as required. Serum samples were collected from four of the patients and were submitted to the Toxicology Service at St George's, University of London for analysis. After 8 hours of observation, none of the patients had evidence of ongoing toxicity. All were discharged home with no long term sequelae, following appropriate drugs education advice.



Figure 2. BZP containing tablets, seized from the nightclub, which were similar to those reportedly ingested by the patients in the ED.

EXPERIMENTAL

Materials

A pure reference sample of N-benzylpiperazine (BZP) was obtained from Fluka Chemie GmbH, Buchs (Switzerland). Quinoline, obtained from BDH (Poole, Dorset, England), was used as an internal standard (IS). Sodium hydroxide (40% solution) and phosphoric acid were also obtained from BDH. HPLC grade methyl-*tert*-butyl-ether (MTBE) was purchased from Rathburn Chemicals Limited (Walkerburn, Scotland).

Sample preparation

The samples were prepared using liquid-liquid extraction. Serum samples (250µL) were adjusted to an alkaline pH with 1M sodium hydroxide (250µL), after the addition of 25µL of the IS quinoline (1mg/L). The solution was extracted with 4mL MTBE. Following centrifugation the organic layer was transferred to 0.1M phosphoric acid (250µL) and mixed. After phase separation by centrifugation the organic layer was removed to waste. 1M sodium hydroxide (100µL) and MTBE (200µL) were added to the remaining subnatent. The samples were then vortex mixed (30 seconds) and centrifuged. An aliquot of the supernatant was injected onto the GC-MS system.

Gas chromatography-mass spectrometry

GC-MS analysis was performed using a Shimadzu GC-MS-QP2010 with a Shimadzu AOC-201 autosampler. A 30 metre, HP-5 MS ((5%-Phenyl)-methylpolysiloxane) column (Agilent, Palo Alto, California), with a 0.253mm i.d. and 0.50µm film thickness was employed for separation. Helium was used as the carrier gas at a flow rate of 1mL per minute.

The injection volume was 1.0µL and injections were made in splitless mode. The injector was maintained at 225°C and the detector at 200°C. The initial column temperature was set at 80°C and held for 4 minutes. It was then ramped by 20°C a minute up to 260°C and held for a further minute, giving a total run time of 14.0 minutes. Positive Electron Impact Ionisation (EI) mode was used and data were collected using single ion monitoring (SIM). BZP and the IS quinoline were identified by their principal ions of 134 and 129 and their retention times of 11.05 and 9.12 minutes respectively.

RESULTS

All samples submitted to the Toxicology Service were negative for alcohol and illicit drugs, including; amfetamines, cocaine, opiates, methadone and benzodiazepines. However BZP was detected in all four samples at concentrations of 1.3, 1.9, 1.9 and 2.5mg/L. Three of these serum concentrations exceeded those reportedly measured in an autopsy case (1.7mg/L).¹ Seizures, such as those witnessed in two of these cases have also been reported by other investigators.³

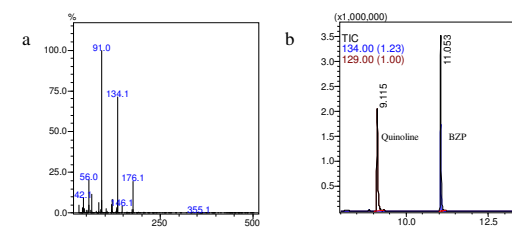


Figure 3. The EI mass spectra (a) and total ion chromatogram (b) produced by BZP.

CONCLUSIONS

We have presented an analytical procedure for the quantitative determination of BZP in serum, by GC-MS. With the popularity of BZP and other piperazines increasing, it is important that toxicology laboratories are aware of this trend and put in place methodology for the routine detection of these compounds.

References:

1. Wikstrom, M. et al. A2 (N-benzylpiperazine) a new drug of abuse in Sweden. *J. Anal Toxicol.* 28: 67-70 (2004).
2. Tekes, K. et al. Studies on the biochemical mode of action of EGYPT-47, a new antidepressant. *Pol. J. Pharmacol Pharm.* 39: 203-211 (1987).
3. Gee, P. et al. Toxic effects of BZP-based herbal party pills in humans: a prospective study in Christchurch, New Zealand. *New Z Med J.* 118:1227 (2005).
4. Bye, C. et al. A comparison of the effects of 1-benzylpiperazine and dexamfetamine on human performance tests. *Eur J Clin Pharmacol.* 6(3): 163-169 (1973).
5. Campbell, H. et al. Comparison of the effects of dexamfetamine and 1-benzylpiperazine in former addicts. *Eur J Clin Pharmacol.* 6(3): 170-176 (1973).
6. Magyar, K. Pharmacokinetic aspects of the mode of action of EGYPT475; a new antidepressant agent. *Pol. J. Pharmacol Pharm.* 39: 107-112 (1987).
7. Baumann, M.H. et al. N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxyamphetamine (MDMA). *Neuropsychopharmacology.* 30(3):550-60 (2005).