



A176 Investigation Into the Stability and Storage Conditions of Benzylpiperazine (BZP) for Chemical Impurity Profiling

Lata Gautam, PhD*, and Beverley R. Vaughan, PhD, Anglia Ruskin University, East Road, Cambridge, CB1 1PT, UNITED KINGDOM; and Michael D. Cole, PhD, Anglia Ruskin University, East Road, Cambridge, CB6 2UD, UNITED KINGDOM

After attending this presentation, attendees will have an increased understanding of the storage and analysis of benzylpiperazine for drug profiling. Beneficiaries will include those in the law enforcement agencies involved in the seizure and analysis of drugs of abuse.

This presentation will impact the forensic science community by increasing the understanding of drug profiling. This presentation will provide a general discussion on handling, storage, and analysis of seized drugs focussing on the latest research and development in this area relating to Benzylpiperazine (BZP).

Since the 1990's a new group of synthetic drugs called "piperazines" have gained popularity among those who use amphetamine type stimulants (ATS) (Staack, 2007).¹ N-benzyl piperazine (BZP), an example of a piperazine type drug was initially synthesized by Wellcome Research Laboratories in the United Kingdom in 1944 as an antiparasitic drug. Its popularity in the illegal drug market is attributed to the physiological effects similar to those of ATS such as "ecstasy" (de Boer et al., 2001) as well as tighter regulatory control on ATS type drugs.² The

number of clinical reports relating to BZP abuse continues to rise, making the development of an analytical method to profile the drug and its clandestine impurities not only necessary but imperative. Until recently, BZP was perceived as a safer legal alternative to ATS. However, since December 2009, BZP has been controlled in the United Kingdom under the Misuse of Drugs Act 1971, Amendment Order 2009. It is also controlled as a Schedule I drug in the United States. There is very little data on optimized analytical and profiling methods for this drug class. This project provides data on the storage conditions and introduction of BZP to an HPLC instrument so that BZP may be successfully analyzed and profiled in support of law enforcement activities.

High performance liquid chromatography (HPLC) was used in this study (Smith et. al., 2008).³ A Shimadzu liquid chromatograph (LC- 10AD VP) with a diode array detector (SIL-10AD VP) fitted with a normal phase silica column (SphereClone 5µm Silica gel, 250 mm x 4.60 mm i.d.) with the detection wavelength of 254 nm was used. The injection volume was 10 µl at 20°C with a flow rate of 1 mL/min and isocratic elution using methanoic HCl (methanol, ammonia, and hydrochloric acid at the ratio of 1000: 9.2: 2.9 v/v), giving a total analysis time of 15 min.

A linear detector response range was established for a drug concentration range between 0.1- 1 mg/mL. The coefficient of correlation (r^2) was found to be 0.99. The limit of detection and limit of quantitation were calculated as 0.426 and 1.15 µg on column respectively.

In order for the data to be valid, the drugs must be stable in the system in which they are introduced to the HPLC. The stability of BZP

(mg/mL) in the solvent used to introduce the drug to the HPLC (methanoic HCl) was investigated in light and dark over a period of 72 hrs. The stability of the BZP solution in the autosampler was also explored. This study shows that the BZP in methanoic HCl is stable at room temperature in conditions of light and dark only up to 22 hrs. The study of the BZP stability in an autosampler revealed similar results.

Having established the analytical conditions necessary for accurate determination of BZP, the next stage was to identify the storage conditions prior to its analysis. BZP was stored under four different conditions: light and dark at room temperature (~20°C), refrigerator (4°C), and freezer (-18°C). Samples were analyzed at time intervals of 0, 24, 48, 72, and 96 hours and the significance of any observed changes were investigated using the Kruskal Wallis non-parametric test at a 95% significance level. The results demonstrate that there was no significant difference between the bench light and dark conditions for 96 hours. However, when samples were stored in an incubator in the dark or in a freezer, analysis showed there was evidence of sample instability.

The conclusions from this study are that BZP samples should be analyzed as soon as possible after seizure and can be stored on a bench in the light or the dark. This study also shows that as an analytical method, the HPLC method presented is suitable for quantitative determination of BZP.

References:

1. Staack, R.F., 2007. Piperazine designer illicit drugs. *Lancet*, 369, pp.1411.
2. de Boer, D., Bosman, I.J., Hidvegi, E., Manzoni C, Benko, A.A., dos Reys, L.J. and Maes, R.A., (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic science international*. 121(1-2), pp.47-56.
3. Smith E. C., Lawther, G., and Cole, M., (2008). Analysis of piperazines by high



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performance liquid chromatography. *Global Forensic Science Today*. 7: pp14-22.
BZP, HPLC, Profiling