Science and Justice 55 (2015) 57–62

Contents lists available at ScienceDirect

Science and Justice

journal homepage: www.elsevier.com/locate/scijus

Precursor discrimination of designer drug benzylpiperazine using $\delta^{13}C$ and $\delta^{15}N$ stable isotopes

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ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form 6 August 2014
Accepted 1 September 2014

Keywords:
Isotopic profiling
IRMS
Designer drugs
Benzylpiperazine
Source discrimination

ABSTRACT

Advances in analytical technology and emerging techniques have resulted in the increased exploitation of chemical and isotopic profiling for source linkage/discrimination of illicit drugs for forensic purposes. Although not routinely used for illicit drug investigations, such information has been obtained and its application demonstrated through the use of isotope ratio mass spectrometry (IRMS). There is a solid platform of research available relating to the isotopic analysis of methylenedioxymethamphetamine (MDMA) and methamphetamine (MA), however with the recently flourishing designer drug market it was of interest to examine the isotopic profiles of the popular 'party drug' benzylpiperazine hydrochloride (BZP·HCl). A preliminary analysis of $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios in BZP·HCl products and corresponding synthetic intermediates (piperazine·HCl) synthesized in-house from three different precursor suppliers was conducted using IRMS. Analysis of the $\delta^{13}C$ and $\delta^{15}N$ isotopic data indicated that discrimination and correct grouping of all the intermediates and some of the product samples examined in this study were achievable.

1. Introduction

The ability to discriminate between different sources and/or identify the synthetic or geographical origins of illicit drugs is greatly desired among the forensic community [1]. Gas chromatography–mass spectrometry (GC–MS) is routinely used by forensic chemists for chemical profiling purposes and although the literature is flooded with successful illicit drug profiling applications [2–11] limitations to this approach have been recognised. For example, the higher purity of some clandestine synthesized illicit drugs such as methylamphetamine (MA) can result in few or no detectable impurities limiting the useful information derived from chemical profiling [12]. An emerging technique that has already demonstrated the ability to provide informative data for the aforementioned drug/scenario is isotope ratio mass spectrometry (IRMS). IRMS can provide powerful data for investigating possible links between for example; precursor–origin, precursor–product, and product–clandestine lab or discriminating between; precursors, precursor sources or synthetic route and intermediate or product batches [13–18].

The isotopic composition of a synthetic or semi-synthetic drug is determined by the isotopic composition found within the precursor chemicals and by any fractionation that occurs during an incomplete chemical reaction [18,19] or physical process [20]. Once a molecule is formed, it will retain its natural stable isotope composition until the molecule is decomposed or structurally altered (bonds broken/formed) [21]. Therefore, any initial differences in the stable isotope composition of the precursors, obtained from different sources will be maintained provided (1) there is an exact stoichiometric mix of the contributing precursors and (2) the reaction goes to completion i.e. there are no side reactions or fractionation occurring.

Similar to GCMS, IRMS has limits to its applicability. Although it may be possible to group samples by batch, it may not be possible to source these samples by origin [22]. Fortunately, an increasing amount of research has reported the isotope analysis of in-house synthesized illicit drugs MDMA [17,22] and MA [15,16], providing crucial information by (i) establishing greater understanding of the isotopic changes during

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http://dx.doi.org/10.1016/j.scijus.2014.09.001
1355-0306/

reactions that makes it possible to identify synthetic routes and (ii) increasing the amounts of published data (traceable to SI standards) that allows wider ranging comparisons.

Designer drugs, such as benzylpiperazine hydrochloride BZP·HCl (Fig. 1), have gained popularity as mimics or substitutes for controlled drugs such as MDMA [13]. The isotopic composition of synthetic illicit drugs such as MDMA and BZP·HCl is potentially characteristic of both the starting materials and the synthetic processes employed [23]. The isotope analysis of MDMA has gained interest as a tool for discriminating between different precursor reagent sources or suppliers [2,3,14,22,24].

In this paper the authors present a preliminary investigation into the potential for using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) to distinguish batches of BZP·HCl and the corresponding synthetic intermediates (piperazine·HCl) based on the precursor source (Fig. 2). To mimic a "real life" scenario a series of BZP·HCl batches were generated following a common clandestine procedure obtained from the Internet (Fig. 2) [25]. Eighteen batches of the intermediate and BZP·HCl products were synthesized using precursors purchased from three different companies. BZP·HCl precipitation was performed using a clandestine method adopted from the Internet [26]. The aromatic moiety of the BZP·HCl products was derived from a common source of benzyl chloride and all other parameters i.e., reaction time, temperature and molar equivalents of reagents were constant.

2. Materials and methods

2.1. Materials

BZP·HCl and intermediate samples were synthesized from the precursor reagents piperazine hexahydrate (PH) and piperazine dihydrochloride (PD) plus benzyl chloride. The piperazine precursors were obtained from the 3 sources; Sigma-Aldrich (SA) (PH, U.K.; PD, Switzerland), Alfa Aesar (AA) (PH, U.K.; PD, U.K.) and MP Biomedical (MP) (PH, France.; PD, Ohio, USA). Benzyl chloride was obtained from Sigma-Aldrich (Missouri, U.S.A.). All other reagents were of commercial quality (obtained from Sigma-Aldrich, U.K. or Alfa-Aesar, U.K.) and were used without further purification.

2.2. Synthesis

Six batches of the intermediate (piperazine·HCl) and product (BZP·HCl) were synthesized from three different sources of piperazine precursor suppliers (SA, AA, MP) following a method obtained from the Internet [25]. BZP·HCl synthesis involved mixing equal molar amounts of the precursors PH and PD to form two molar equivalents of the intermediate piperazine·HCl. The intermediate was then reacted with one molar equivalent of the precursor benzyl chloride to form one molar equivalent of BZP base. Approximately 1 molar equivalent of unreacted intermediate was recovered from the reaction mix for IRMS analysis. BZP base was precipitated as the hydrochloride salt (BZP·HCl) following a common clandestine method adopted from the Internet (reported yield range from 93 to 95%) (presented in Fig. 2) [25,26].

Fig. 1. BZP containing 'party pills'.

2.3. Nuclear magnetic resonance spectroscopy

^{1}H and ^{13}C NMR spectra were obtained using a Bruker Biospin 300 MHz spectrometer (Bruker Biospin AG, Switzerland) at 300 and 75 MHz, respectively.

2.4. Low resolution mass spectrometry

Mass spectral analysis was achieved using a Bruker Esquire 3000 electrospray ionization ion trap mass spectrometer (Bruker Daltonic GmbH, Germany).

2.5. Gas chromatography–mass spectrometry

GC–MS was carried out using an Agilent GC–MS with electron ionization (EI) set at 70 eV. The identification and purity of in-house synthesized BZP were determined by GC–MS performed in the following conditions: Agilent Ultra Inert column DB-5MS (30 m × 0.25 mm film thickness 0.25 μm); carrier gas, He (1.0 mL min^{-1}); splitless; MS temperature, 250 °C. Elution was by thermal gradient under conditions; 80 °C (4 min)/20 °C min^{-1} to 280 °C (8 min/20 °C min^{-1}) to 290 °C for 11.5 min. The retention time of BZP was 9.6 min. Samples were prepared in ethyl acetate (1 mg/mL).

Samples were characterized by GC–MS, NMR and MS analyses. Representative spectra are presented in the Supporting Information.

2.6. Isotope ratio mass spectrometry

2.6.1. Bulk ^{13}C and ^{15}N isotope analysis

Triplicate samples of each BZP·HCl product and the corresponding intermediate were prepared for carbon and nitrogen isotopic analyses by weighing a small amount (0.05–0.15 mg) into tin capsules and then pelletizing. Isotope data were acquired by combusting the samples at 1050 °C in a Sercon Europa EA-GSL elemental analyser. The CO_2, NO_x and H_2O produced during the combustion were carried in a helium flow through a reduction reactor filled with copper at 650 °C to reduce NO_x to N_2 then through an Anhydrone® trap to remove water. CO_2 and N_2 gases were chromatographically separated and fed into a Sercon Hydra 20–22 isotope mass spectrometer where the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ from the solid samples were obtained. Measured isotope abundance values were normalised to the international reporting scales, Vienna Pee Dee Belemnite (VPDB) (^{13}C) and Air (^{15}N), by 2-point calibration using contemporaneously analysed reference materials (RMs) obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria): IAEA-CH-6 ($\delta^{13}C_{VPDB} = -10.45‰$) and IAEA-CH-71 ($\delta^{13}C_{VPDB} = -32.15‰$) for carbon and IAEA-N1 ($\delta^{15}N_{AIR} = +0.43‰$) and IAEA-N2 ($\delta^{15}N_{AIR} = +20.35‰$) for nitrogen [27]. In addition, in-house standards of sucrose and ammonium sulfate were analysed in duplicate throughout the analytical sequence as quality assurance.

3. Results and discussion

The $\delta^{13}C$ and $\delta^{15}N$ values for the precursors are presented in Table 1 and the $\delta^{13}C$ and $\delta^{15}N$ data for the intermediates and products are presented in Table 2. The range of $\delta^{13}C$ and $\delta^{15}N$ obtained for the intermediates derived from the three precursor sources was $\delta^{13}C_{VPDB} = -26.8$ to $-30.1‰$ and $\delta^{15}N_{AIR} = +3.6$ to $+8.7‰$. The intermediates were essentially a combination of the precursors PH/PD,