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Analytical challenges in the confirmative identification of dipyrone as an adulterant in illicit drug samples

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ABSTRACT

Dipyrone is an analgesic and antipyretic drug that is sometimes encountered as an adulterant in illicit drug samples, particularly illicit fentanyl containing samples. It undergoes thermal decomposition to aminopyrine and 4-methylaminoantipyrine during analysis via gas chromatography (GC–FID) and gas chromatography–mass spectrometry (GC–MS). During analysis via high pressure liquid chromatography (HPLC) and high pressure liquid chromatography–mass spectrometry (HPLC–MS), it undergoes hydrolytic decomposition solely to 4-methylaminoantipyrine. Given that mass spectrometry is a widely used confirmatory analytical technique, these instabilities present challenges for the forensic chemist seeking to confirm the presence of dipyrone. Studies were conducted to determine rigorous confirmative protocols for the identification of dipyrone in multicomponent illicit drug samples.

1. Introduction

To the extent that it is technically feasible and economically practical, illicit drugs are typically manufactured and enter the upper echelons of the drug distribution chain in states of high chemical purity. In the case of illicit drugs distributed in powder form, by the time they emerge for retail sale and consumption at the street level of the drug distribution chain, they have typically been cut with a variety of unrelated licit powders as a profit boosting measure. Such cutting agents may be classified as either diluents or adulterants. A diluent is typically a pharmacologically inactive agent that dilutes the originally pure illicit drug serving only to add bulk. An adulterant not only serves a similarly dilutive role, but also imparts specific pharmacological activity, which may or may not be similar to the psychoactive effect of the illicit drug. As a result of these practices, an illicit drug initially manufactured with high chemical purity is oftentimes significantly reduced in chemical purity by the time it reaches the end user.

Historically, for street level seizures of heroin submitted to this laboratory for forensic analysis, sugars such as lactose and mannitol are examples of commonly identified diluents. Examples of adulterants commonly identified in such samples include diphenhydramine, caffeine, quinine, acetaminophen and lidocaine. Recently, this laboratory encountered street level heroin samples adulterated with fentanyl, a potent synthetic opioid receptor agonist. In fact, upon forensic analysis, some seizures purported to contain heroin as the controlled substance, were found to contain fentanyl rather than heroin, or sometimes a mixture of both. In the United States, heroin is a Schedule I controlled substance and fentanyl is a Schedule II controlled substance. These classifications notwithstanding, fentanyl is estimated to be about 30–50 times more potent than heroin [1]. The adulteration of heroin with fentanyl is not a new phenomenon. During the period from April 4th, 2005 through March 28th, 2007, the United States Centers for Disease Control and Prevention (CDC) documented a total of 1013 deaths which were directly attributed to either fentanyl or heroin that had been adulterated with fentanyl [2,3]. However, the fact that recently seized illicit fentanyl containing samples tend to contain the rather rare adulterant dipyrone (which is also known generically as metamizole in some countries), is a new development.

Dipyrone (Fig. 1) is an analgesic and antipyretic drug. Although it was withdrawn from the US market in 1977 due to safety concerns [4], and is similarly prohibited in other countries, it is still widely available, either as prescription only with restrictions in some countries, or over the counter (OTC) in a number of other countries [5]. It is possible that its use as a fentanyl adulterant is analogous to the prevalent use of other analgesics (e.g. acetaminophen, ibuprofen and aspirin) as adulterants in illicit drug samples.

In this laboratory, provided that the testing scheme employed includes a separatory technique, forensic analytical identification of any component (whether controlled or non-controlled) in a

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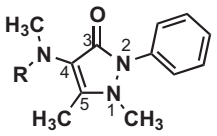

R = CH_2SO_3Na	Dipyrone
R = CH_2SO_3H	Dipyrone Acid
R = CH_3	Aminopyrine
R = H	4-Methylaminoantipyrine

Fig. 1. Structure of dipyrone and related compounds including systematic numbering of the core pyrazolone ring.

suspected illicit drug sample, typically involves a minimum of one presumptive and one confirmative test. The structure of dipyrone imparts certain physical and chemical characteristics that may complicate meeting these requirements. This paper will delineate and demonstrate these complications and offer solutions for addressing them.

2. Materials and methods

2.1. Chemicals and standards

All general use chemicals, reagents and solvents were purchased either from Fisher Scientific (Fair Lawn, NJ, USA) or Sigma–Aldrich Inc. (St. Louis, MO, USA) and used as received without further purification. 4-Methylaminoantipyrine was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). All other reference standards were obtained from this laboratory's drug reference standard collection.

2.2. Instrumentation and methods

2.2.1. Gas chromatography–mass spectrometry (GC–MS)

GC–MS experiments were conducted using an Agilent Technologies (Santa Clara, CA) Model 7890A gas chromatograph (GC) equipped with a DB-5 MS capillary column (30 m × 250 μm × 0.25 μm) and interfaced with an Agilent Model 5975C mass selective detector (MSD). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV and a scan range of 40–500 amu. Samples were prepared without regard to concentration in a 4:1 mixture of chloroform and methanol containing 0.0125 mg/mL of tetracosane as an internal standard. The GC injector was operated in the split mode (10–60:1 ratio, depending on sample strength). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injection port temperature was set to 280 °C and the transfer line was held at 280 °C. The oven temperature was programmed as follows: initial temperature held at 150 °C for 1.5 min, then ramped at 30 °C/min to a final temperature of 310 °C and held isothermal for 8.16 min. Instruments equipped with HP-5 MS columns were used for analyses of the dipyrone reference standard as indicated in the results and discussion sections. These column choices were based on instrument availability at the time of analysis.

2.2.2. Gas chromatography–flame ionization detection (GC–FID)

GC–FID experiments were conducted on an Agilent Technologies (Santa Clara, CA) Model 7890A gas chromatograph (GC) equipped with a DB-5 capillary column (12 m × 200 μm × 0.33 μm). Samples were prepared at a concentration of approximately 10 mg/mL in a 4:1 mixture of chloroform and methanol containing 0.0125 mg/mL of tetracosane as an internal standard. The injector was operated in the split mode (60:1 ratio). Nitrogen was used as the carrier gas at a constant flow rate of 1 mL/min. The injection port temperature was set to 270 °C and the detector was set at 310 °C. The oven temperature was programmed as follows: initial temperature held at 165 °C for 1 min, then ramped at 15 °C/min to 280 °C, held for 1 min, then ramped at 30 °C/min to a final temperature of 310 °C and held isothermal for 2.5 min.

2.2.3. High pressure liquid chromatography (HPLC)

HPLC experiments were conducted using a Waters Acquity Ultra Performance LC system equipped with a binary pump, an HSS T3 column (1.8 μm, 2.1 × 100 mm) and a photo diode array detector. Detection was achieved at 210 nm. Mobile phase A (buffer) was 20 mM sodium phosphate, pH 2.3, with 0.2% hexylamine and 1.5 mM sodium azide. Mobile phase B was acetonitrile. A gradient method was programed as follows—flow rate: 0.5 mL/min; initial mobile phase: 99.5% mobile phase A/0.5% mobile phase B; initial hold: 0.90 min; Gradient Program 1: mobile phase composition was linearly ramped to 80% mobile phase A for 3.1 min; hold time 0.0 min; Gradient Program 2: mobile phase composition was linearly ramped to 65% mobile phase A for 1.0 min; hold time 0.35 min. The injection solvent consisted of 95:5 buffer/acetonitrile with 0.2 mg/mL of theophylline-7-acetic acid as an internal standard. Samples were ground to a homogenous powder and approximately 10 mg of sample was dissolved in 1.0 mL of the injection solvent. All solutions were filtered through a 0.2 μm polytetrafluoroethylene (PTFE) or polyvinylidene difluoride (PVDF) syringe filter prior to analysis. A concentration of approximately 1 mg/mL was used for all reference standard solutions. After filtering, one drop of concentrated (i.e. 85%) *o*-phosphoric acid was added to each sample, which was then mixed thoroughly.

2.2.4. Electrospray ionization mass spectrometry (ESI-MS)

ESI-MS experiments were conducted using a Thermo Finnigan (San Jose, CA) LCQ mass spectral detector equipped with a syringe infusion pump and an electrospray ionization (ESI) probe. Samples were ground to a homogenous powder and approximately 1 mg of sample was dissolved in 10 mL of methanol. A concentration of approximately 10 μg/mL was used for all reference standard solutions.

2.2.5. Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra were recorded on a Varian (Palo Alto, CA, USA) Mercury Plus 400 MHz NMR spectrometer. Analytes were dissolved in DMSO-d_6 (dimethylsulfoxide) containing tetramethylsilane (TMS) as the internal standard and filtered when necessary.

3. Results and discussion

3.1. Background

Fig. 2 shows the total ion chromatogram (TIC) obtained during the gas chromatography–mass spectrometry (GC–MS) analysis (i.e. confirmatory testing) of a suspected heroin containing illicit drug sample **1**.

No heroin was detected. The only controlled substance detected was fentanyl (retention time, RT 8.17 min). With the instrument conditions used, heroin typically elutes at about RT 7.95 min. All remaining peaks in the chromatogram were ascribed to non-controlled constituents (i.e. adulterants or diluents). The major peak at RT 8.76 min was determined to be the adulterant quinine. The peak at 5.15 min was caffeine and the peak at 7.85 min was suspected to be cinchonidine. The peak at RT 6.81 min is an added internal reference standard, tetracosane. Of the remaining peaks of significant intensity, the mass spectrum of the peak at RT 5.47 min was consistent with aminopyrine (Fig. 1). Using the National Institute of Standards and Technology (NIST) version 11 and