Forensic Chemistry 9 (2018) 50–61

Contents lists available at ScienceDirect

Forensic Chemistry

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

Mass spectral differentiation of positional isomers using multivariate statistics

Jennifer Bonetti

Virginia Department of Forensic Science, Eastern Laboratory, 830 Southampton Avenue, Suite 400, Norfolk, VA 23510, United States

ARTICLE INFO

Article history:
Received 20 February 2018
Received in revised form 7 May 2018
Accepted 4 June 2018
Available online 05 June 2018

Keywords:
Designer drugs
Positional isomers
Multivariate analysis
GC–MS

ABSTRACT

Mass spectral analysis is considered to be a confirmatory method of analysis for the purpose of identifying controlled substances. However, the spectra of positional isomers are generally too visually similar to allow for differentiation. This study of fluoromethcathinone (FMC) and fluorofentanyl has shown that multivariate statistical analysis offers a feasible means of differentiating between electron ionization mass spectra of positional isomers. Three positional isomers of each compound (2-FMC, 3-FMC, 4-FMC, *meta*-fluorofentanyl, *ortho*-fluorofentanyl, and *para*-fluorofentanyl) were analyzed twice daily using gas chromatography/electron ionization mass spectrometry (GC/EI-MS) on six separate instruments over five days. This resulted in 60 mass spectra collected for each positional isomer. Principal component analysis (PCA) followed by linear discriminant analysis (LDA) was performed and successful differentiation of positional isomers was achieved. Leave-One-Sample-Out Cross Validation showed no errors for either group of isomers. Nineteen blind study samples were analyzed for each group of positional isomers with no misclassifications. Furthermore, data from previous case samples was analyzed using this method and in all cases the samples were properly attributed to the correct positional isomer. In addition to visual inspection of the LDA plots, objective classifications were conducted using the resulting posterior probabilities generated when LDA was performed. The results indicate multivariate statistical analysis is a promising addition to the analytical scheme of the identification of positional isomers. This would allow for higher confidence in the final identification of a compound without the need for additional instrumental analysis, saving laboratories both time and money.

1. Introduction

In all fields of forensic science, analysts must have confidence in their conclusions and the ability to defend their findings in a court of law. Forensic drug analysis is a discipline where this task is not generally considered a hardship because it is based on a strong analytical chemistry foundation. It is due to this foundation that the field of drug analysis has avoided much of the scrutiny bestowed upon other forensic disciplines by reports such as those by the National Academy of Sciences (NAS) in 2009 and the President's Council of Advisors on Science and Technology (PCAST) in 2016 [1,2]. However, forensic drug chemists should always seek to continuously improve their discipline. As new designer drugs are frequently introduced whose structures differ from those of existing controlled substances solely by the addition/subtraction of various chemical moieties, it has become necessary to expand the standard analytical approach and develop new methods of spectral comparison to assist with identification [3]. One area that still presents a challenge for forensic drug chemists is the differentiation between positional isomers. Although the precise technical definition varies, a positional isomer is widely considered to be a compound that shares the same molecular formula and general structural backbone with another substance but differs in the specific placement of certain functional groups or substitutions [4,5]. This high level of similarity means positional isomers are often not easily discriminated using the most common forensic techniques [6].

In federal and state legislative codes, positional isomers are often controlled at the same level as the listed compound [7–10]. Therefore, when a controlled substance is identified, isomer determination is generally not conducted. If there is a known common isomer, the substance may be reported as the listed compound "or one of its isomers."[11] While this practice meets all essential legal requirements, it gives reports an unnecessary air of ambiguity. In addition, pharmacological studies have shown that positional isomerism can have an effect on the potency and biological effect of a drug [12,13]. For instance, one of the positional isomers of carfentanil (3-carbomethoxy fentanyl, or, *iso*-carfentanil) is found to

E-mail address: Jennifer.Bonetti@dfs.virginia.gov

https://doi.org/10.1016/j.forc.2018.06.001
2468-1709/

be about 60 times less potent than carfentanil itself [14]. It is clear that the ability to easily differentiate between a dangerous drug and its positional isomers is of paramount importance.

One of the primary instrumental techniques for the confirmation of the identity of controlled substances is Gas Chromatography/Mass Spectrometry (GC/MS) [15]. Due to their inherent structural similarities, most positional isomers produce mass spectra that are visually similar to one another. While there are some positional isomers that exhibit Gas Chromatography (GC) retention time differences depending on the column and method utilized, this cannot be said for all isomers [6,16–19]. In some instances, a derivatization procedure can be combined with GC/MS analysis to differentiate between isomers [16,20]. However, derivatization can be a time-consuming step, and the most appropriate derivatization technique can change based on which compound is being analyzed.

There are several other instrumental methods available to differentiate between positional isomers. However, many of the currently available methods, such as Nuclear Magnetic Resonance Spectroscopy (NMR) and Fourier-Transform Infrared Spectroscopy (FTIR), require a relatively pure sample, something that is not routinely encountered in forensic casework. The time required to extract and purify a sample in order to perform these techniques is often not feasible given the immense caseload threatening to overwhelm forensic laboratories. In addition, neither technique is suitable for the analysis of extremely small samples such as the residue remaining on suspected paraphernalia. To address some of these limitations of isomer determination with the use of FT-IR and NMR, both instruments can be coupled with a gas chromatograph to isolate a compound prior to analysis [21,22], but many laboratories cannot justify the expense. There have been other suggested methods for the differentiation of isomers but they generally require time-consuming analysis, or additional, often expensive, instrumentation [23–26]. Due to these limitations, there exists a need to develop an efficient method that can confidently discriminate between positional isomers using data that is already generated during the course of routine analysis.

The use of multivariate statistics to differentiate between electron ionization mass spectra of positional isomers has been previously published [27,28]. One study investigated the relatively simple mass spectra generated by the isomers of xylene using principal component analysis (PCA). With this method, the authors were able to demonstrate the ability of PCA to discriminate between visually indistinguishable mass spectra [27].

The application of multivariate statistics has also been investigated for a wide variety of forensic disciplines such as counterfeit bill determination [29], trace evidence analysis [30–32], and drug analysis for the determination of the synthetic route of fentanyl samples [33]. PCA has been utilized in the study of positional isomers of controlled substances, specifically cannabimimetic agents [34]. However, in that instance, PCA was applied to only five of the most abundant mass fragments rather than to the entire spectrum. This approach was primarily utilized as an investigative tool to determine which ions forensic analysts should focus on for the differentiation of the specific isomers studied. It was not suggested that analysts incorporate the multivariate methods themselves into the analytical procedure. The current study investigates the feasibility of utilizing multivariate techniques as a means of positional isomer identification as part of the forensic identification scheme.

Two groups of positional isomers were studied for this preliminary investigation: fluoromethcathinone (FMC), also sometimes referred to as fluoro-*N*-methylcathinone, and fluorofentanyl. Three positional isomers for each of these compounds all reflect changes in the position of the fluorine on the aromatic ring (Fig. 1). The Code of Federal Regulations specifically lists 3-FMC and 4-FMC in §1308.11 (d) as Schedule I substances. The description of this section states that the salts, isomers, and salts of isomers of the listed compounds will be considered equivalent for scheduling purposes. For this section, it is specified that "the term "isomer" includes the optical, position and geometric isomers" which indicates that 2-FMC is also considered a Schedule I substance at the federal level [8]. Para-fluorofentanyl is the only fluorofentanyl isomer that is specifically listed in §1308.11 (b) as a Schedule I substance. This section includes isomers as well, but the term in this section is limited to optical isomers only, and does not include positional isomers. An emergency scheduling action was enacted which added *ortho*-fluorofentanyl as a temporary Schedule I substance from October 26, 2017 to October 28, 2019. On February 6, 2018, another temporary scheduling action was enacted which controls *meta*-fluorofentanyl by considering all "Fentanyl-related substances" to be Schedule I substances until February 6, 2020 [8].

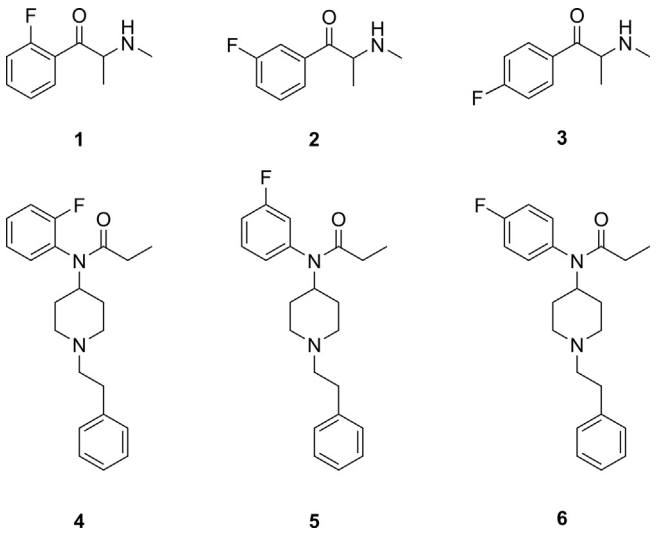

Fig. 1. Structures of the positional isomers studied. 1 = 2-fluoromethcathinone. 2 = 3-fluoromethcathinone. 3 = 4-fluoromethcathinone. 4 = Ortho-fluorofentanyl. 5 = Meta-fluorofentanyl. 6 = Para-fluorofentanyl.

During the time in which the *ortho*-fluorofentanyl and *meta*-fluorofentanyl isomers were not considered Schedule I substances at the federal level, they were still considered controlled under the definition of a controlled substance analogue listed in the Controlled Substances Act [35]. However, successful prosecution of a crime related to a controlled substance analogue has other provisions that must be met, so it is clear that the identification of the specific positional isomer would be crucial to determining whether those provisions are necessary or if the compound is a Schedule I substance itself based solely on its identity. The language used for these compounds may differ from state to state, so in some states all six of the positional isomers studied are considered Schedule I substances and the specific positional isomer identification would not be required for successful prosecution.

2. Materials and methods

2.1. Standard preparation

Primary standards of the fluorofentanyl isomers, as well as 2-FMC and 3-FMC, were obtained from Cayman Chemical, (Ann Arbor, MI) while the primary standard for 4-FMC was obtained from Lipomed, Inc. (Cambridge, MA). All standard solutions were prepared in GC-Grade methanol (Honeywell Burdick & Jackson, Muskegon, MI) at concentrations of 1.5 mg/mL and 0.5 mg/mL for FMC and fluorofentanyl, respectively. The difference in concentration was