B54 The Characterization and Differentiation of 21 Fentanyl Analogues by Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Mass Spectrometry (LC/MS), and Nuclear Magnetic Resonance (NMR) Spectroscopy

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After attending this presentation, attendees will gain a deeper knowledge regarding the analysis of fentanyl analogues. This analysis includes the separation and identification of the structural and geometric isomers of fentanyl analogues and the unusual behaviors exhibited by several fentanyl analogues evaluated by NMR spectroscopy.

This presentation will impact the forensic science community by providing the analytical data of 21 fentanyl analogues, including the structural and geometric isomers, by GC/MS, LC/MS, and NMR spectroscopy.

Fentanyl is a powerful synthetic analgesic developed in the 1960s. To date, a wide variety of fentanyl analogues, such as α-methylfentanyl and 3-methylfentanyl, have been abused all over the world. In the present study, 21 compounds of fentanyl analogues, including structural and geometric isomers, were analyzed by GC/MS, LC/MS, and NMR spectroscopy.

GC/MS conditions: column, capillary column coated with 5% phenyl methylpolysiloxane (30m × 0.25mm i.d.); oven temperature, 120°C (1min hold) and programmed up to 300°C at a rate of 15°C/min; injection port temperature, 250°C; carrier gas, helium; sample injection, splitless mode; ionization, Electron Ionization (EI); electron energy, 70 eV. LC/MS conditions: column, ODS column (2.1 × 150mm); mobile phase composition, 10mM ammonium acetate (A) and methanol (B); linear gradient mode, 80% A and 20% B to 10% A and 90% B over 18min, followed by a 2min hold at 10% A and 90% B, and returned to 80% A and 20% B in 0.1min; flow rate, 0.20ml/min; MS interface, positive Electrospray Ionization (ESI). NMR conditions: proton resonance frequency, 600MHz; solvent, $CDCl_3$ or CD_3OD; internal standard, tetramethylsilane.

In GC/MS, fentanyl analogues, except for fentanyl and acetyl-α-methylfentanyl, were separated on the Extracted Ion Chromatograms (EIC) of the characteristic fragment ions of each compound. Fentanyl and acetyl-α-methylfentanyl were separated by slowing the rate of the temperature increase of the column to 3°C/min. Geometric isomers (*cis-trans* isomers) of 3-methylfentanyl analogues were completely separated by GC. The thermal decomposition of β-hydroxyfentanyl should be closely monitored: a high-injection port temperature (>250°C) induced the cleavage of the C-N bond of β-hydroxyfentanyl to form norfentanyl. The thermal decomposition of β-hydroxyfentanyl was completely suppressed by Trimethylsilyl (TMS) derivatization of the hydroxyl group.

In LC/MS, most of the fentanyl analogues were separated on the EICs of the protonated molecule of each compound. It is notable that the two diastereomers of β-hydroxy-*cis*-3-methylfentanyl were separated by LC, but not by GC; however, it was difficult to separate the diastereomers of β-hydroxy-*trans*-3-methylfentanyl by GC and LC in this study.

Several 3-methylfentanyl analogues exhibited unusual behavior when analyzed by ^{1}H-NMR. Specifically, the

proton signal of the 3-methyl group of acetyl-*cis*-3-methylfentanyl hydrochloride split at a ratio of 3:2, when this compound was dissolved in $CDCl_3$. The same phenomenon was observed in the cases of *cis*-3-methylfentanyl hydrochloride and *cis*-3-methylthiofentanyl hydrochloride. In contrast, when these compounds were dissolved in CD_3OD, the proton signal did not split. This phenomenon may be explained as follows: the proton of the hydrochloride coordinated to the nitrogen of the piperidine ring when the hydrochloride salts of the 3-methylfentanyl analogues were dissolved in a non-polar solvent, such as $CDCl_3$. A pair of stereoisomers were formed by the coordination, and the split of the proton signal of the 3-methyl group was observed.

Fentanyl, Isomer, Analysis