Identification of Isobaric Methyl-Substituted Fentanyl Analogs Using a Transportable Linear Ion Trap Mass Spectrometer

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INTRODUCTION

Fentanyl and fentanyl analogs are at the center of the ongoing opioid epidemic in the United States. Even though fentanyl is a Schedule II controlled substance due to its accepted medicinal use, fentanyl analogs are Schedule I controlled substances due to their significant risk to public health, with hundreds of overdoses occurring daily [1,2]. Consequently, there is growing interest in the development of rapid in-field techniques for the identification of fentanyl analogs. One potential solution is the application of field-portable techniques, such as color tests and immunoassays. However, these techniques struggle to provide the necessary selectivity and sensitivity to identify fentanyl analogs [3]. In comparison, transportable mass spectrometry instrumentation provides more characteristic information about unknown samples analyzed in the field, especially for instrumentation capable of tandem mass spectrometry (MS/MS) analysis.

In this study, a method was developed for the identification of isobaric methyl-substituted fentanyl analogs using a transportable linear ion trap mass spectrometer. The eight analogs analyzed in this study included a methyl substituent at common locations of substitution to the core fentanyl structure. Each analog was characterized based on the resulting product ion spectra. Validation studies were completed to establish selectivity, repeatability, reproducibility, and the limit of detection (LOD). Finally, the efficacy of this method was evaluated through the analysis of methyl-substituted fentanyl analogs present in two- and three-component mixtures.

MATERIALS & METHODS

Chemicals and materials

Eight methyl-substituted fentanyl analogs representative of different R-group substitutions were analyzed in this study.

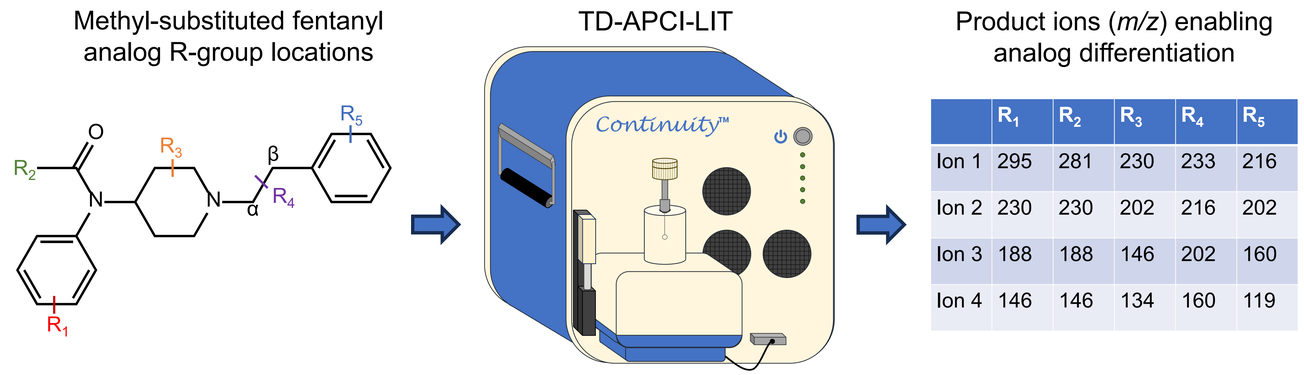
Table 1. Methyl-substituted fentanyl analogs in this study.

R Group	Methyl-Substituted Fentanyl Analog
R_1	Ortho-methyl fentanyl
R_2	Butyryl fentanyl Isobutyryl fentanyl
R_3	Trans-3-methyl fentanyl 4-Methyl fentanyl
R_4	α-Methyl fentanyl β-Methyl fentanyl
R_5	4'-Methyl fentanyl

Sample Preparation

Pure, 2-, and 3-component mixtures were analyzed in this study with a concentration of 20 ppm per analog in a 49.9%:49.9%:0.02% MeOH:H_2O:acetic acid solution. All analogs were purchased as certified reference materials from Cayman Chemical or Cerilliant.

RESULTS & DISCUSSION



	R_1	R_2	R_3	R_4	R_5
Ion 1	295	281	230	233	216
Ion 2	230	230	202	216	202
Ion 3	188	188	146	202	160
Ion 4	146	146	134	160	119

Figure 1. Overview of the developed method for identifying methyl-substituted fentanyl analogs.

- Methyl substituted fentanyl analogs were analyzed using TD-APCI-LIT to obtain MS/MS product ion spectra.
- Characteristic product ions enable the differentiation between R-group substitution locations.

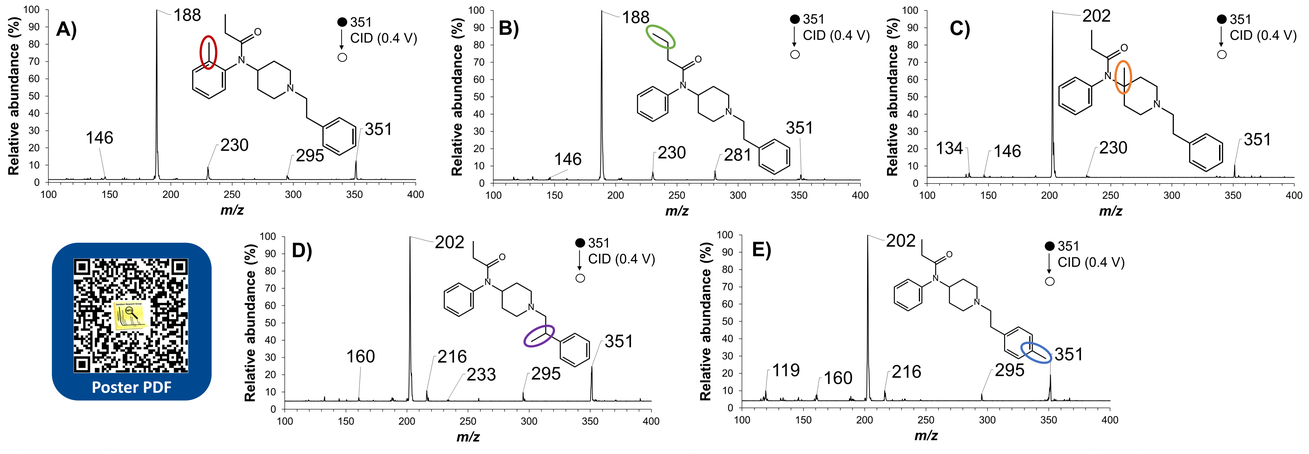


Figure 2. Exemplar product ion spectra of a methyl-substitution at each R-group location: A) ortho-methyl fentanyl (R_1), B) butyryl fentanyl (R_2), C) 4-methyl fentanyl (R_3), D) β-methyl fentanyl (R_4), and E) 4'-methyl fentanyl (R_5).

- Differences in the MS/MS product ion spectra are observed due to different methyl substitution locations to the core fentanyl structure.

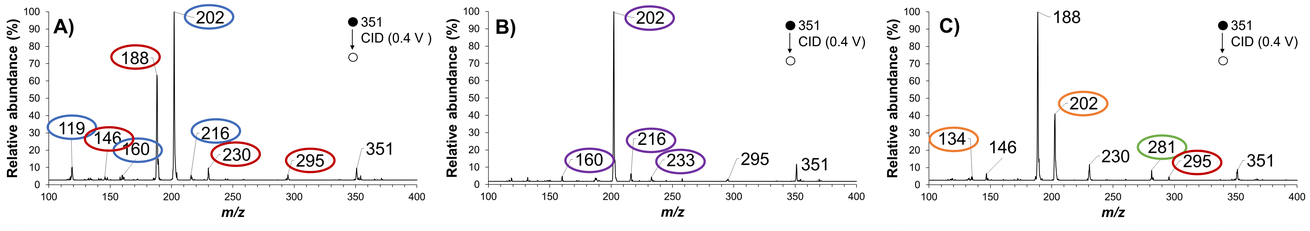


Figure 3. Exemplar product ion spectra of two- and three-component mixtures: A) ortho-methyl fentanyl (R_1) + 4'-methyl fentanyl (R_5), B) β-methyl fentanyl (R_4) + α-methyl fentanyl (R_4), and C) ortho-methyl fentanyl (R_1) + butyryl methyl fentanyl (R_2) + trans-3 methyl fentanyl (R_3).

- Using the characteristic ions for the different R-group substitution locations, it is possible to identify different methyl-substituted fentanyl analogs within two- and three-component mixtures.
- If the mixture is composed of methyl-substituted fentanyl analogs with the same substitution location, the analogs cannot be differentiated.

MATERIALS & METHODS

A thermal desorption atmospheric pressure chemical ionization (TD-APCI) source coupled to a Continuity™ linear ion trap (LIT) transportable mass spectrometer was used to analyze all samples. MS/MS product ion spectra were collected using an RF level: 200 V, CID frequency center: 89 kHz, CID amplitude: 0.4 V, and detector anode: -1150 V. The Library ID list to MS/MS mode with a threshold of 3000 counts was utilized to collect MS/MS data. The resulting data was exported to Microsoft Excel for data visualization. Ions enabling isomer differentiation and the corresponding MS/MS parameters were imported into the internal library.

Validation Studies

The validation studies were assessed for all methyl-substituted fentanyl analogs as follows; selectivity: analyzing both pure and mixture samples (n=3), repeatability: n=5 within one day, reproducibility: n=5 for 4 days over two weeks, and LOD: serial dilution until the Library ID list to MS/MS mode was no longer triggered.

CONCLUSIONS

- Eight methyl-substituted fentanyl analogs were characterized using a transportable linear ion trap mass spectrometer.
- An internal library was developed for the identification of the methyl-substituted fentanyl analogs.
- Validation studies revealed a repeatable and reproducible method with an LOD of 5 ppm.
- Differentiation between R-group substitutions is possible based on differences within MS/MS product ion spectra.
- MS/MS product ion spectra for compounds of the same R-group substitution location cannot be differentiated.
- R-group substitution location was identifiable in mixtures composed of different R-group substitutions.

REFERENCES

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