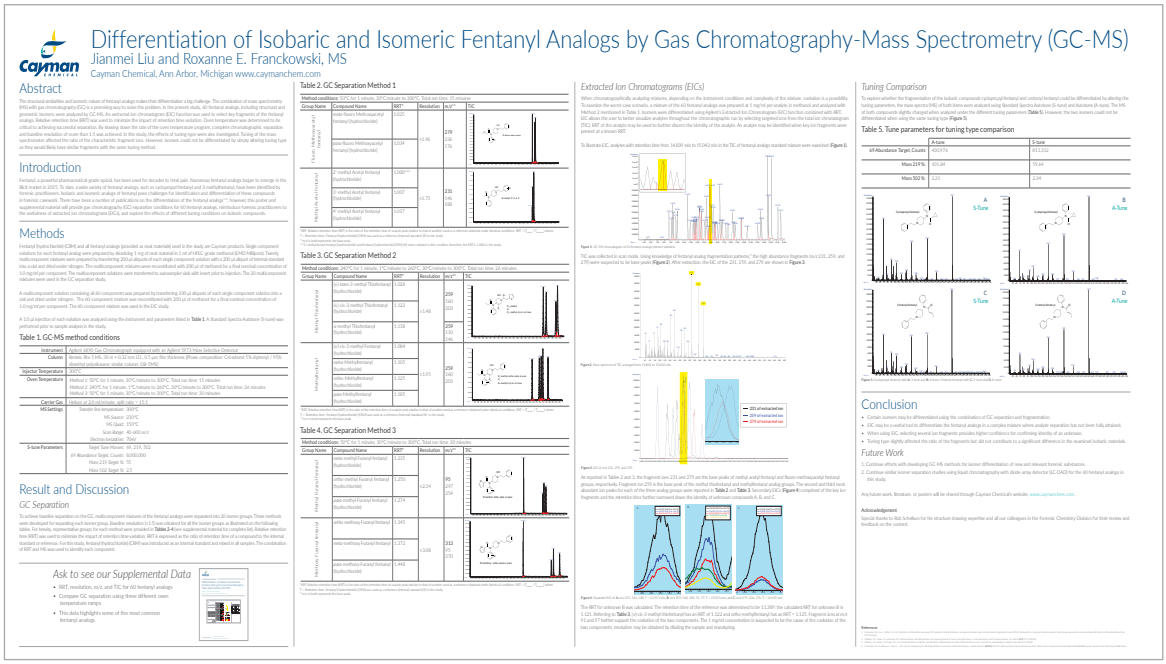
SUPPLEMENTAL MATERIAL

Differentiation of Isobaric and Isomeric Fentanyl Analogs by Gas Chromatography-Mass Spectrometry (GC-MS)

Jianmei Liu and Roxanne E. Franckowski, MS
Cayman Chemical, Ann Arbor, Michigan www.caymanchem.com



Differentiation of Isobaric and Isomeric Fentanyl Analogs by Gas Chromatography-Mass Spectrometry (GC-MS)

Jianmei Liu and Roxanne E. Franckowski, MS

Cayman Chemical, Ann Arbor, Michigan www.caymanchem.com

Abstract

Introduction

Methods

Result and Discussion

GC Separation

Ask to see our Supplemental Data

Table 1. GC-MS method conditions

Table 2. GC Separation Method 1

Table 3. GC Separation Method 2

Table 4. GC Separation Method 3

Extracted Ion Chromatograms (EICs)

Tuning Comparison

Table 5. Tune parameters for tuning type comparison

Conclusion

Future Work

Cayman Chemical · (800) 364-9897
1180 E. Ellsworth Road · Ann Arbor, MI · 48108

GC Separation Methods

Fentanyl (hydrochloride) (CRM) and all fentanyl analogs (provided as neat materials) used in the study are Cayman products. Single component solutions for each fentanyl analog were prepared by dissolving 1 mg of neat material in 1 ml of HPLC-grade methanol (EMD Millipore). Twenty multicomponent mixtures were prepared by transferring 200 µl aliquots of each single component solution with a 200 µl aliquot of internal standard into a vial and dried under nitrogen. The multicomponent mixtures were reconstituted with 200 µl of methanol for a final nominal concentration of 1.0 mg/ml per component. The multicomponent solutions were transferred to autosampler vials with insert prior to injection. The 20 multicomponent mixtures were used in the GC separation study.

A 1.0 µl injection of each solution was analyzed using the instrument and parameters listed in **Tables 1-4.** A Standard Spectra Autotune (S-tune) was performed prior to sample analysis in the study.

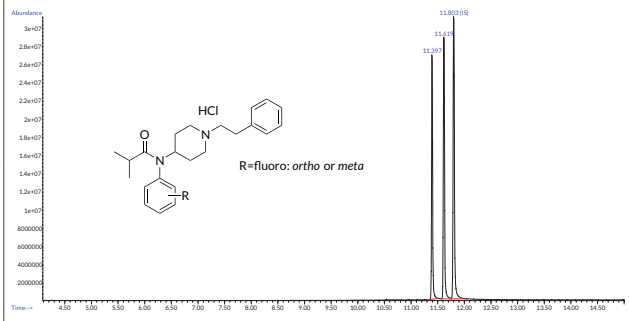
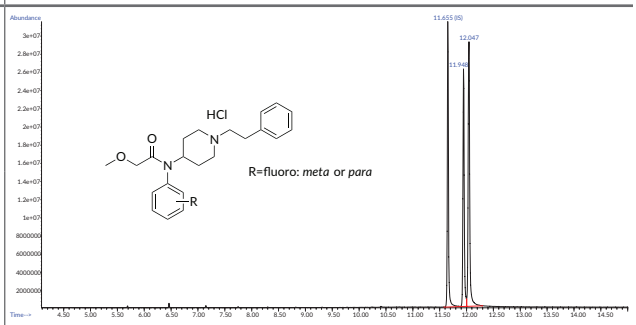
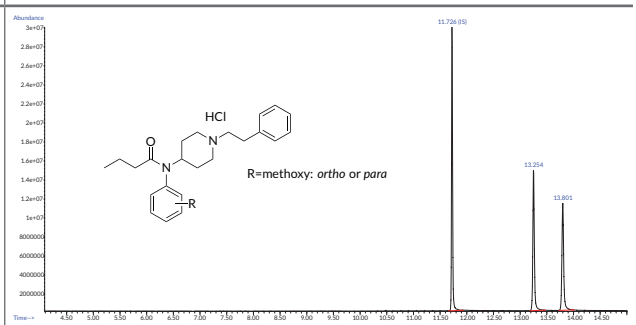
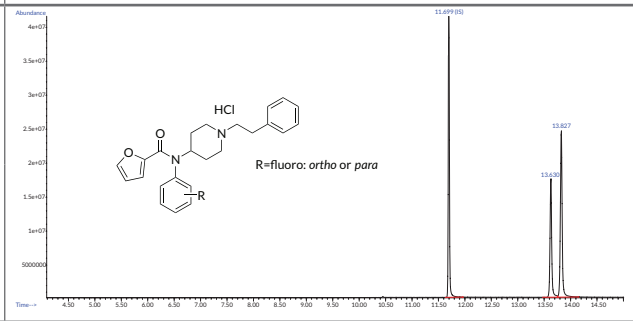
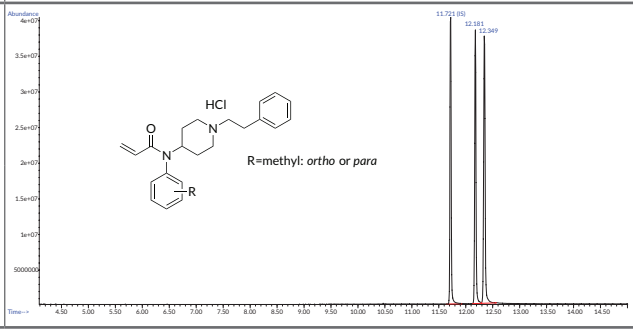
Table 1. GC-MS method conditions

Instrument	Agilent 6890 Gas Chromatograph equipped with an Agilent 5973 Mass Selective Detector
Column	Restek, Rtx-5 MS, 30 m × 0.32 mm I.D., 0.5 µm film thickness (Phase composition: Crossbond 5% diphenyl / 95% dimethyl polysiloxane; similar column: DB-5MS)
Injector Temperature	300°C
Carrier Gas	Helium at 2.0 ml/minute, split ratio = 15:1
MS Settings	*Transfer line temperature:* 300°C
	MS Source: 230°C
	MS Quad: 150°C
	Scan Range: 40-600 *m/z*
	Electron Ionization: 70eV
S-tune Parameters	*Target Tune Masses:* 69, 219, 502
	69 Abundance Target, Counts: 8,000,000
	Mass 219 Target %: 55
	Mass 502 Target %: 2.5

To achieve baseline separation on the GC, multicomponent mixtures of the fentanyl analogs were separated into 20 isomer groups. Three methods were developed for separating each isomer group. Baseline resolution (≥1.5) was obtained for all the isomer groups as illustrated in **Tables 2-4**. Results for each method are listed in the tables below. Relative retention time (RRT) was used to minimize the impact of retention time variation. RRT is expressed as the ratio of retention time of a compound to the internal standard or reference. For this study, fentanyl (hydrochloride) (CRM) was introduced as an internal standard and mixed in all samples. The combination of RRT and MS was used to identify each component.

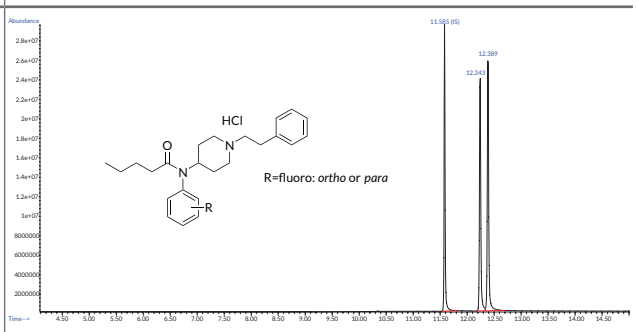
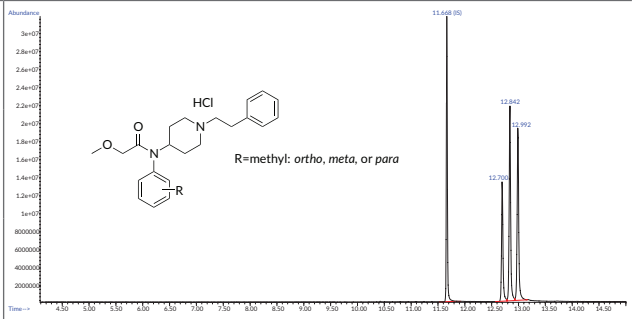
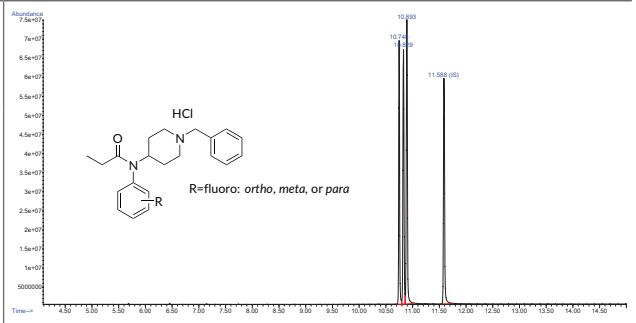
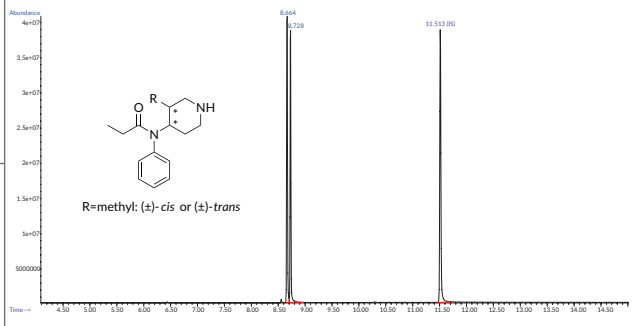
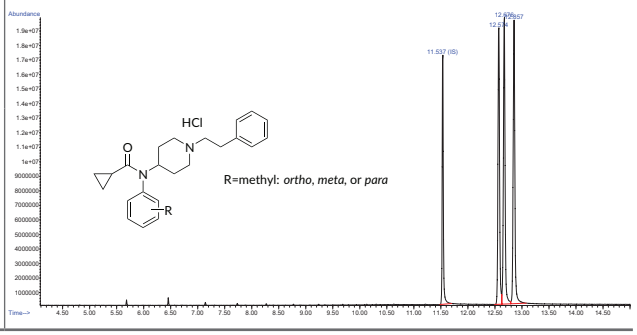
Table 2. GC Separation Method 1

Method conditions: 50°C for 1 minute, 30°C/minute to 300°C. Total run time: 15 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Fluoroisobutyryl fentanyl	*meta*-Fluoroisobutyryl fentanyl (hydrochloride)	20206	0.966	≥5.28	**277** 207 164	
	ortho-Fluoroisobutyryl fentanyl (hydrochloride)	20207	0.984			
Fluoro Methoxyacetyl fentanyl	*meta*-fluoro Methoxyacetyl fentanyl (hydrochloride)	21740	1.025	≥1.96	**279** 236 176	
	para-fluoro Methoxyacetyl fentanyl (hydrochloride)	21741	1.034			
Methoxy Butyryl fentanyl	*ortho*-methoxy Butyryl fentanyl (hydrochloride)	22035	1.130	≥9.81	**289** 176 219	
	para-methoxy Butyryl fentanyl (hydrochloride)	18089	1.177			
Fluoro Furanyl fentanyl	*para*-fluoro Furanyl fentanyl (hydrochloride)	22750	1.165	≥3.22	**95** 301 258	
	ortho-fluoro Furanyl fentanyl (hydrochloride)	23628	1.182			
Methyl Acrylfentanyl	*ortho*-methyl Acrylfentanyl (hydrochloride)	23036	1.039	≥3.37	**257** 160 214	
	para-methyl Acrylfentanyl (hydrochloride)	23038	1.054			

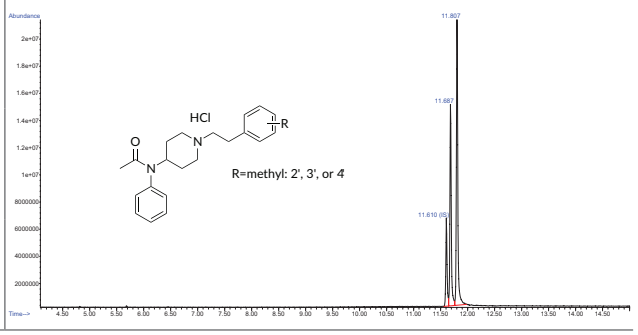
GC Separation Method 1 *(continued)*

Method conditions: 50°C for 1 minute, 30°C/minute to 300°C. Total run time: 15 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Fluoro Valeryl fentanyl	*para*-fluoro Valeryl fentanyl (hydrochloride)	23085	1.057	≥3.04	**291** 164 207	
	ortho-fluoro Valeryl fentanyl (hydrochloride)	23083	1.069			
Methyl Methoxyacetyl fentanyl	*meta*-methyl Methoxyacetyl fentanyl (hydrochloride)	22978	1.088	≥2.82	**275** 232 172	
	ortho-methyl Methoxyacetyl fentanyl (hydrochloride)	22977	1.101			
	para-methyl Methoxyacetyl fentanyl (hydrochloride)	22979	1.113			
N-benzyl fluoro Norfentanyl	N-benzyl *meta*-fluoro Norfentanyl (hydrochloride)	25541	0.928	≥1.56	**91** 82 172	
	N-benzyl *para*-fluoro Norfentanyl (hydrochloride)	25542	0.935			
	N-benzyl *ortho*-fluoro Norfentanyl (hydrochloride)	25540	0.940			
3-Methyl Norfentanyl	(±)-*trans*-3-methyl Norfentanyl	22695	0.753	≥1.99	**98** 97 82	
	(±)-*cis*-3-methyl Norfentanyl	22617	0.758		**93** 97 82	
Methyl Cyclopropyl fentanyl	*ortho*-methyl Cyclopropyl fentanyl (hydrochloride)	23043	1.090	≥1.80	**271** 160 203	
	meta-methyl Cyclopropyl fentanyl (hydrochloride)	23044	1.099			
	para-methyl Cyclopropyl fentanyl (hydrochloride)	23045	1.114			

GC Separation Method 1 *(continued)*

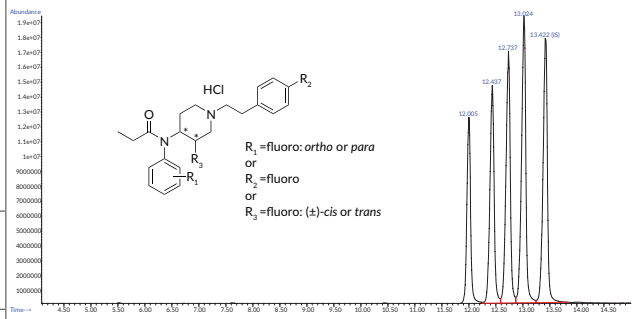
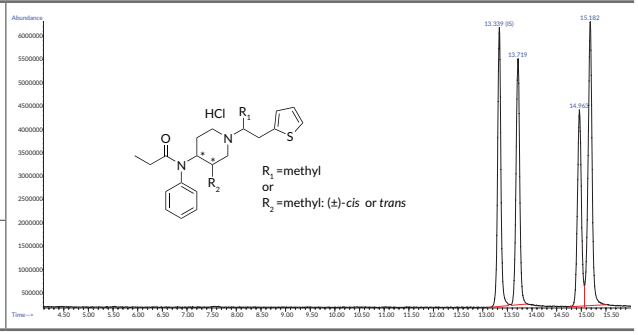
Method conditions: 50°C for 1 minute, 30°C/minute to 300°C. Total run time: 15 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Methyl Acetyl fentanyl	2'-methyl Acetyl fentanyl (hydrochloride)	25473	1.000***	≥1.73	**231** 146 188	
	3'-methyl Acetyl fentanyl (hydrochloride)	25474	1.007			
	4'-methyl Acetyl fentanyl (hydrochloride)	9002271	1.017			

*RRT: Relative retention time (RRT) is the ratio of the retention time of analyte peak relative to that of another used as a reference obtained under identical conditions. RRT = ($T_{analyte}$ / $T_{reference}$) where T = Retention time. Fentanyl (hydrochloride) (CRM) (**Item No. ISO60197**) was used as a reference (internal) standard (IS) in the study.
m/z in **bold represents the base peak.
***2'-methyl Acetyl fentanyl (hydrochloride) and fentanyl (hydrochloride) (internal standard) were coeluted in this condition, therefore, the RRT is 1.000 in the study.

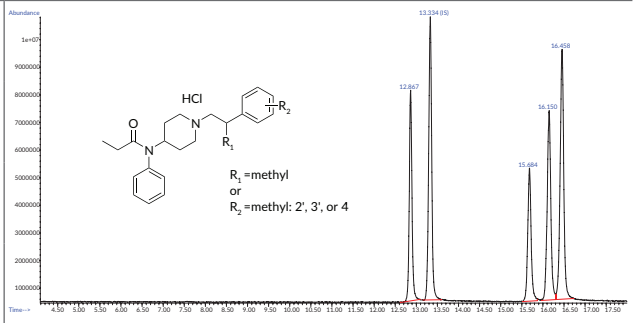
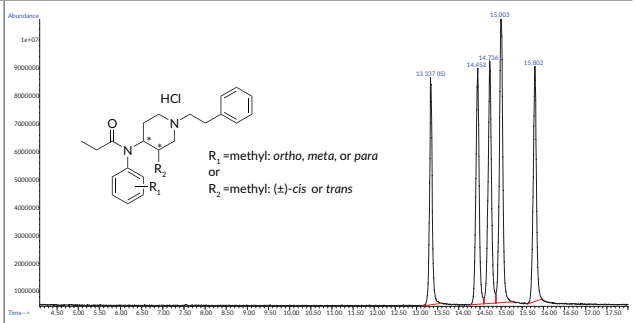
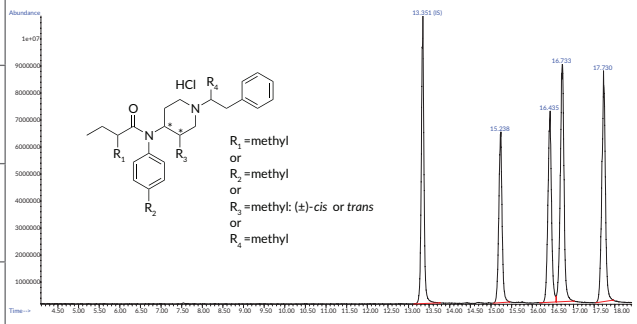
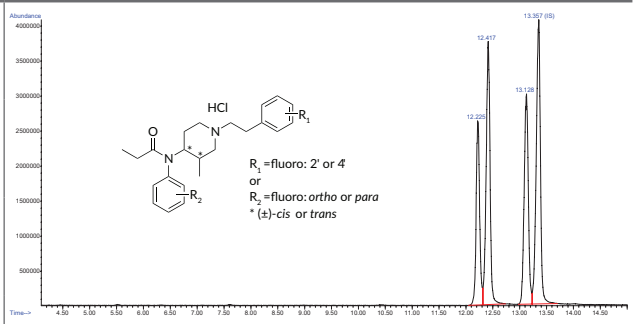
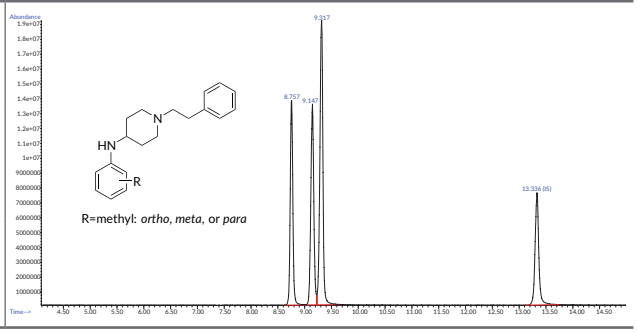
Table 3. GC Separation Method 2

Method conditions: 240°C for 1 minute, 1°C/minute to 260°C, 30°C/minute to 300°C. Total run time: 26 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Fluorofentanyl	*meta*-Fluorofentanyl (hydrochloride)	19424	0.894	≥2.16	**263** 164 207	
	para-Fluorofentanyl (hydrochloride)	15496	0.927			
	ortho-Fluorofentanyl (hydrochloride)	19425	0.949			
	4'-Fluorofentanyl (hydrochloride)	25877	0.970		**245** 146 189	
	3-Fluorofentanyl (hydrochloride)	21952	1.000***		**263** 207 186	
Methyl Thiofentanyl	(±)-*trans*-3-methyl Thiofentanyl (hydrochloride)	22241	1.028	≥1.48	**259** 160 203	
	(±)-*cis*-3-methyl Thiofentanyl (hydrochloride)	22240	1.122			
	α-methyl Thiofentanyl (hydrochloride)	20821	1.138		**259** 110 146	

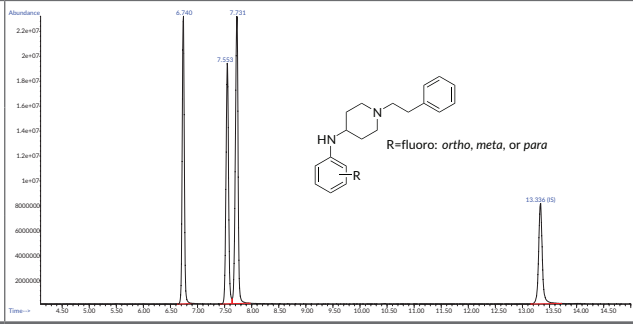
GC Separation Method 2 *(continued)*

Method conditions: 240°C for 1 minute, 1°C/minute to 260°C, 30°C/minute to 300°C. Total run time: 26 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Methylfentanyl	β-methyl Fentanyl (hydrochloride)	9002860	0.965	≥1.97	**245** 146 189	
	3'-methyl Fentanyl (hydrochloride)	25476	1.176			
	4'-methyl Fentanyl (hydrochloride)	21914	1.211			
	2'-methyl Fentanyl (hydrochloride)	25475	1.234			
	(±)-*cis*-3-methyl Fentanyl (hydrochloride)	9002747	1.084	≥1.95	**259** 160 203	
	meta-Methylfentanyl (hydrochloride)	22633	1.105			
	ortho-Methylfentanyl (hydrochloride)	22634	1.125			
	para-Methylfentanyl (hydrochloride)	20038	1.185			
Methyl Butyryl fentanyl	α'-methyl Butyryl fentanyl (hydrochloride)	22994	1.141	≥2.21	**273** 189 146	
	(±)-*cis*-3-methyl Butyryl fentanyl (hydrochloride)	20817	1.231		**273** 160 203	
	α-methyl Butyryl fentanyl (hydrochloride)	20818	1.253		**273** 91 146	
	para-methyl Butyryl fentanyl (hydrochloride)	23048	1.328		**273** 160 203	
Fluoro substituted 3-Methylfentanyl	2'-fluoro, *ortho*-fluoro (±)-*trans*-3-methyl Fentanyl (hydrochloride)	24312	0.915	≥1.79	**277** 178 109	
	4'-fluoro, *ortho*-fluoro (±)-*trans*-3-methyl Fentanyl (hydrochloride)	24511	0.930			
	2'-fluoro, *ortho*-fluoro (±)-*cis*-3-methyl Fentanyl (hydrochloride)	24311	0.983			
	4'-fluoro, *para*-fluoro (±)-*cis*-3-methyl Fentanyl (hydrochloride)	24502	1.000***			
Despropionyl Methylfentanyl	Despropionyl *ortho*-Methylfentanyl	24279	0.657	≥1.68	**160** 203 294	
	Despropionyl *meta*-Methylfentanyl	25744	0.686			
	Despropionyl *para*-Methylfentanyl	25745	0.699			

GC Separation Method 2 *(continued)*

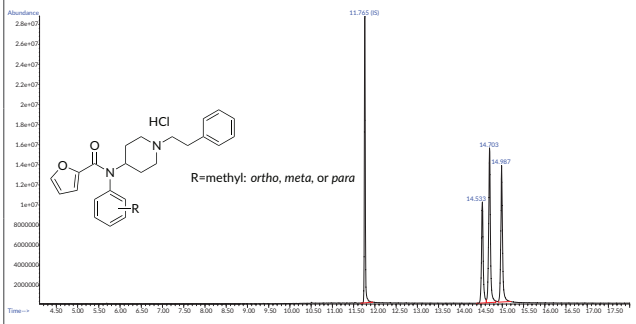
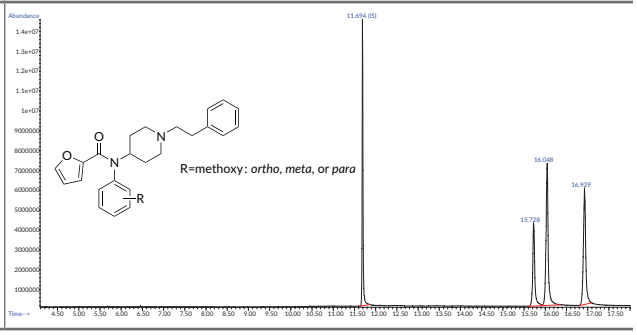
Method conditions: 240°C for 1 minute, 1°C/minute to 260°C, 30°C/minute to 300°C. Total run time: 26 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Despropionyl Fluorofentanyl	Despropionyl *ortho*-Fluorofentanyl	20126	0.505	≥1.96	**164** 207 91	
	Despropionyl *para*-Fluorofentanyl	20104	0.566			
	Despropionyl *meta*-Fluorofentanyl	20125	0.580			

*RRT: Relative retention time (RRT) is the ratio of the retention time of analyte peak relative to that of another used as a reference obtained under identical conditions. RRT = ($T_{analyte}$ / $T_{reference}$) where T = Retention time. Fentanyl (hydrochloride) (CRM) (**Item No. ISO60197**) was used as a reference (internal) standard (IS) in the study.
m/z in **bold represents the base peak.
***3-Fluorofentanyl (hydrochloride); 4'-fluoro, *para*-fluoro (±)-*cis*-3-methyl fentanyl (hydrochloride) and fentanyl (hydrochloride) (internal standard) were coeluted in this condition, therefore, the RRT is 1.000 in the study, respectively.

Table 4. GC Separation Method 3

Method conditions: 50°C for 1 minute, 30°C/minute to 300°C. Total run time: 30 minutes

Group Name	Compound Name	Cayman Item No.	RRT*	Resolution	*m/z***	TIC
Methyl Furanyl fentanyl	*meta*-methyl Furanyl fentanyl (hydrochloride)	22844	1.235	≥2.34	**95** 297 254	
	ortho-methyl Furanyl fentanyl (hydrochloride)	22779	1.250			
	para-methyl Furanyl fentanyl (hydrochloride)	22713	1.274			
Methoxy Furanyl fentanyl	*ortho*-methoxy Furanyl fentanyl	22805	1.345	≥3.80	**95** 313 270	
	meta-methoxy Furanyl fentanyl	26050	1.372			
	para-methoxy Furanyl fentanyl (hydrochloride)	23197	1.448			

*RRT: Relative retention time (RRT) is the ratio of the retention time of analyte peak relative to that of another used as a reference obtained under identical conditions. RRT = ($T_{analyte}$ / $T_{reference}$) where T = Retention time. Fentanyl (hydrochloride) (CRM) (**Item No. ISO60197**) was used as a reference (internal) standard (IS) in the study.
m/z in **bold represents the base peak.