Rapid-field-deployable DART-MS screening technique for 87 opioids and drugs of abuse, including fentanyl and fentanyl analogs

Sara E. Kern[a, 1], Valerie M. Toomey[a], Lisa M. Lorenz[a]

[a] *U. S. Food and Drug Administration, Office of Regulatory Affairs, Forensic Chemistry Center, Cincinnati, OH USA*

Abstract

Fentanyl and fentanyl analogs are playing an increasing role in the opioid health crisis that is sweeping America. These synthetic opioids are much more potent and lethal than morphine or heroin and must be treated with extreme caution. Many of these substances are entering the country through International Mail Facilities (IMFs) and Express Courier Hubs (ECHs) in the form of counterfeit prescription drugs and bulk powders, resulting in a critical need for field-deployable techniques that can safely provide rapid screening to prevent these products from reaching the public. Here we describe a direct analysis in real time thermal desorption mass spectrometer (DART-MS) with database searching capabilities that allows quick detection of numerous target compounds. Minimum detectable levels for 87 opioids and drugs of abuse have been determined. This technique was applied to eight samples sent to the Food and Drug Administration's (FDA) Forensic Chemistry Center for analysis and the results are compared to those collected using gas chromatography with mass spectral detection (GC-MS) and liquid chromatography with mass spectral detection (LC-MS).

Keywords: ambient ionization, mass spectrometry, field deployable instrumentation, opioids

Introduction

The opioid health crisis has evolved over two decades, with rising overdose deaths from synthetic opioids becoming more prevalent than deaths due to heroin or prescription opioids. Drug overdose deaths in the United States rose 28.5% to an estimated 100,306 during a twelve month period ending in April, 2021, including 75,673 involving opioids, according to preliminary data released by the Centers for Disease Control and Prevention, as compared to the same time span during the previous year. [1,2] According to the Drug Enforcement Agency (DEA), many of these substances are coming from other countries, particularly China, and entering through international mail facilities (IMFs) and express courier hubs (ECHs). [4] Most IMFs and ECHs are not equipped with laboratory facilities or experienced analysts, creating a need for field-deployable equipment that is fast, safe, and facile to use. The Food and Drug

[1] [1] Corresponding author: Sara E. Kern, Email: sara.kern@fda.hhs.gov

Administration (FDA) is increasing the number of inspectors, analysts, and detection capabilities at IMFs and ECHs to help prevent opioids from entering the United States. [5].

Spectroscopic methods such as handheld Raman spectrometers, which can safely examine samples through clear glass and plastic, [9,3] have been used extensively in the field, but this technique performs best with relatively pure or highly concentrated samples, which is often not true of products containing synthetic opioids. [7] Fourier transform infrared spectrometers are popularly used in the field, but they are also limited by their lack of sensitivity. [8,14] A more sensitive and field-tested technique is ion mobility spectrometry, but this method is limited in selectivity since it uses only drift time for identification of analytes. [16]

One technique that is both sensitive and selective is direct analysis in real time ionization coupled to a thermal desorption unit interfaced with a mass spectrometer (DART-MS). DART-MS is a relatively established technique that has been demonstrated to work well in a variety of forensic applications, including trace levels of drugs of abuse on the outside of packages, both in laboratory and field settings [10, 11, 12, 13]. Recently, this ionization source was coupled to a rugged and simple single quadrupole mass analyzer and successfully evaluated by the FDA as part of a portable analytical toolkit to analyze various active pharmaceutical ingredients in drug products. [6] This toolkit has been deployed at a satellite laboratory at the Chicago IMF to help increase the total number of packages examined and screened for the presence of opioids. [5] DART-MS has also been used in combination with a portable Raman instrument to screen seized drugs. [2]

The DART ionization source eliminates the need for chromatography and drastically decreases the time, effort and supplies required for sample preparation. The instrument has a thermal desorption unit that encloses the swab safely to prevent inhalation of the desorbed sample, a crucial feature when analyzing substances such as fentanyl and fentanyl analogs that are lethal in small doses. The instrument and computer may be fully contained on a movable cart and requires a standard 110 V power supply and high purity nitrogen for ionization. A searchable, expandable mass spectral database allows data processing so that manual mass spectral interpretation is not necessary.

This study provides minimum detectable levels on the order of nanograms for 87 opioids and drugs of abuse, including fentanyl and fentanyl analogs, using a DART-MS method and applies the method to eight samples that were sent to the FDA's Forensic Chemistry Center for analysis. While these particular samples were sent to a brick-and-mortar laboratory for evaluation, it demonstrates the versatility of this technique when faced with an unusual matrix or a limited sample size. The types of samples encountered at ports of entry vary greatly and are not always pharmaceutical products, so it is important to have the ability to analyze many types of items.

Methods and Materials

Reagents and Supplies

Noscapine, papaverine, methamphetamine hydrochloride, and heroin standards were obtained from Sigma-Aldrich (St. Louis,

MO, USA). A standard of 6-monoacetylmorphine hydrochloride trihydrate was acquired from Grace Davison Discovery Sciences (Epping, Australia). A standard of 6-acetylcodeine was purchased from Cerilliant (Round Rock, TX, USA) and all other standards were obtained from Cayman Chemicals (Ann Arbor, MI, USA). All standards were purchased in their neat form, either as solids or liquids. HPLC grade acetonitrile, methanol, formic acid, and polytetrafluoroethylene (PTFE) filters were purchased from Fisher Scientific (Hampton, NH, USA). Ultra-high purity nitrogen (>99.9%) was acquired from American Welding and Gas (Raleigh, NC, USA). Sample traps (PTFE coated fiberglass, also referred to as "swabs", catalog # ST1322P) were obtained from DSA Detection (Boston, MA, USA).

Samples

Personnel from the FDA/ORA Office of Criminal Investigations collected eight samples from a dumpster outside of a facility suspected of manufacturing illicit drugs and sent them to the FDA's Forensic Chemistry Center for analysis (see Figure 1 for images of the samples). The samples included a section of newspaper with blue stains, multiple paper towels and disposable wipes with blue and brown stains, two yellow surgical masks with brown stains, multiple portions of clear plastic bags, and a portion of bubble wrap with duct tape. These samples were subdivided by the lead analyst. For the LC-MS and GC-MS experiments, portions of the items were submerged in 2.0 mL of methanol, vortexed briefly, sonicated for 10 minutes, and shaken using a mechanical shaker for 10 minutes. The disposable wipes and face masks were also centrifuged at 3500 rpm for 5 minutes. A syringe with a 0.2 µm PTFE filter was used to isolate the extraction solvent. The filtrate from the newspaper and face masks was further diluted 1:100 , the filtrate from the disposable wipes and bags was further diluted 1:20 and the filtrate from the bubble wrap and duct tape was further diluted 1:10 with 50% acetonitrile/reverse osmosis deionized water (RODI) water for the LC-MS analyses. For the GC-MS analyses, the filtrate from the newspaper, disposable wipes, one face mask, and some of the bags were further diluted 1:10 with methanol. The filtrate from one of the masks and some of the bags were further diluted 1:3 with methanol and the filtrate from the bubble wrap with duct tape was diluted 1:1 with methanol. The DART-MS sampling involved swabbing a single DART sample trap (DSA Detection) on the stained portions of the newspaper, disposable wipes, surgical face mask, plastic bag, and bubble wrap (with duct tape) samples.

Instrument Methods

DART-MS

The DART-MS experiments were performed using a DART-SVP 100 model interfaced with a thermal desorption (TD) unit (Ionsense, Inc., Saugus, MA, USA) coupled to a Waters (Milford, MA, USA) QDa single quadrupole mass detector. Data were acquired using Mass Lynx V4.2 software from Waters and then processed using NextGenPIMISA® V1.0 software from IonSense. The DART was operated in positive mode with the nitrogen temperature set to 300°C and the grid voltage set to 300 V. The TD unit was set to 250°C. The QDa was operated with a positive polarity, using four cone voltages (15, 30, 50, 70 V) to collect in-source collision induced dissociation

(is-CID) fragmentation. Mass spectra were acquired over the range *m/z* 50-1050 with an acquisition time of 20 seconds and a scan rate of 5 Hz.

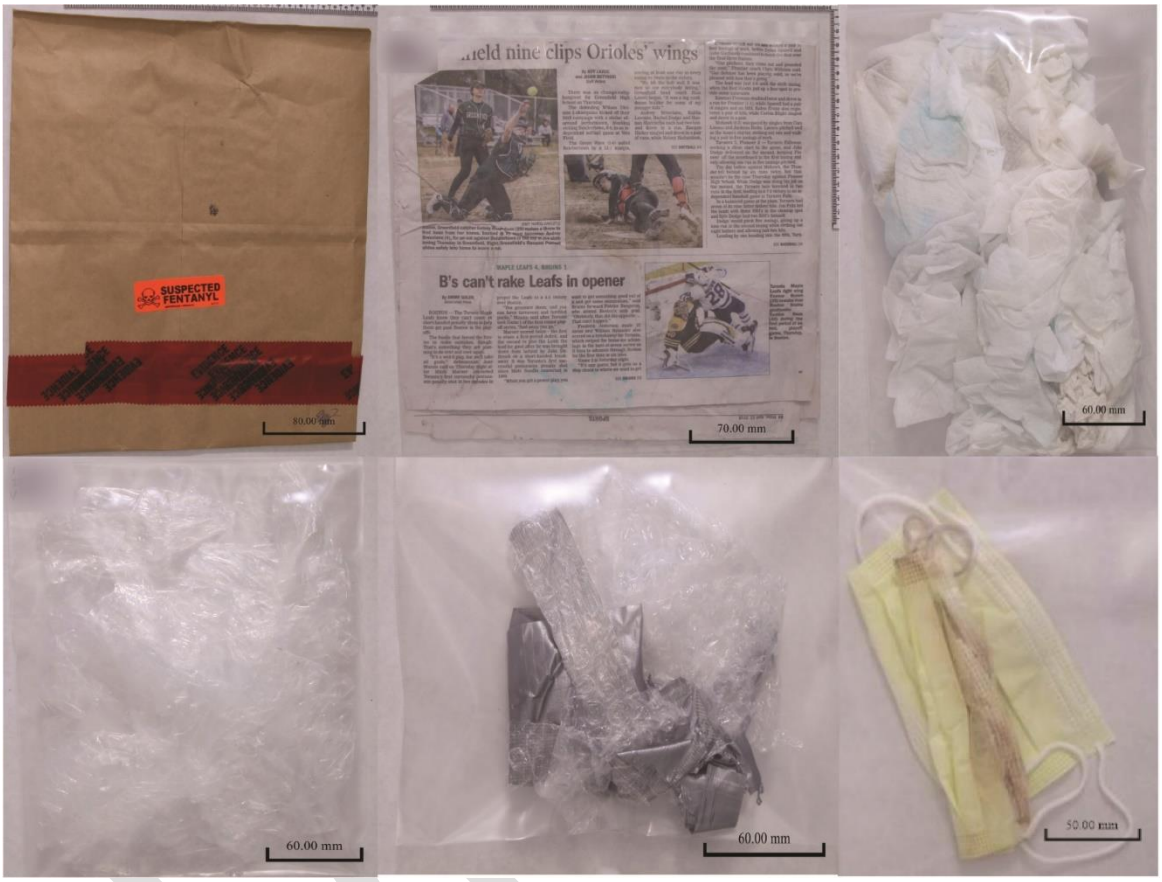

Figure 1: Images of the samples submitted for analysis.

Standard solutions of approximately 1000 ug mL^{-1} were prepared in acetonitrile. Subsequent dilutions were prepared and 4 uL aliquots were pipetted onto sample traps such that 5 ng increments of standard were applied. The minimum detectable level was determined by analyzing each standard in triplicate and processing the data files using the NextGenPIMISA® V.1.0 software. A database match of 90% or greater for the triplicate swabs was required for the minimum detectable level and for acceptable sample results. Blank sample traps were run in between each standard and sample to ensure there was no carry-over.

LC-MS

LC-MS analyses were performed using a ThermoScientific Velos Pro mass spectro-

meter with a Thermo ESI source coupled to a ThermoScientific Vanquish liquid chromatograph. A C18 Agilent Eclipse Plus column with dimensions of 2.1 mm x 50 mm, 1.8 µm, maintained at 40 °C, was utilized. A gradient from 5% to 50% B over 4 minutes, then 50% to 90% B over one minute with a hold from 5 to 7 minutes, using 0.1% formic acid in 18.2 MΩ·cm reverse osmosis deionized (RODI) water (A) and 0.1% formic acid in acetonitrile (B), were employed at a flow rate of 0.35 mL/min for a run time of 7.0 minutes. A post-run equilibration time of 2.0 minutes was employed to return the system to the initial conditions. The autosampler was maintained at ambient temperature or 15 °C, with an injection volume of 1 µL.

Data acquisition was accomplished using Thermo Xcalibur v.4.0.27.19 software, with the following mass spectrometer parameters: positive electrospray ionization; sheath gas flow of 45 arbitrary units, auxiliary gas flow of 5 arbitrary units, sweep gas flow of 3 arbitrary units, spray voltage of 3.0 kV; capillary temperature of 370 °C. Three scan events were used to collect the MS^n data. The first scan event collected the full scan MS data over the range *m/z* 90-900. The second scan event was a data-dependent scan to collect MS/MS data on the most intense ions from a precursor mass list using a collision energy of 40% and the third scan event was a data-dependent scan to collect MS^3 data on the most intense product ion from the second event using a collision energy of 35%. Standard solutions of approximately 1-10 ug mL^{-1} were prepared in 50% acetonitrile/RODI water.

GC-MS

GC-MS analyses were performed using a Thermo TRACE 1310 Series GC with an AI 1310 Series autosampler coupled to an ISQLT Thermo Mass Selective Detector (MSD). A Phenomenex Zebron AB-5HT Inferno column with dimensions of 35 meters, ID 250 µm, and a film thickness of 0.25 µm was utilized. The flow rate was 1.2 mL/min, with an injection temperature of 250 °C, an injection volume of 1.0 µL, in split and splitless modes. The initial temperature was 75 °C with a hold time of 1.00 minute and a ramp rate of 10 °C/min to a final temperature of 300 °C and holding for 10.0 minutes. The MS acquisition parameters included a solvent delay of 4.5 minutes, electron impact ionization, a scan range of *m/z* 40-550, an MSD source temperature of 280 °C, and a total run time of 33.50 minutes. Data acquisition was accomplished using Xcalibur 3.0.63 software. Standard solutions of approximately 200-400 ug mL^{-1} were prepared in methanol.

Table 1. Opioids and illicit drug standards analyzed to determine minimum detectable levels (MDL) using DART-MS (Note – the MDL is calculated by the free base).

Compound	**CAS#**	**Formula (free base)**	**$[M+H]^+$ (*m/z*) (free base)**	**Fragment Ions (*m/z*)**	**MDL, ng**
Methamphetamine HCl	51-57-0	$C_{10}H_{15}N$	150	91, 65	10

NPP	39742-60-4	$C_{13}H_{17}NO$	204	202, 134, 105, 103, 79, 77	5
Acetyl norfentanyl HCl	22352-82-5	$C_{13}H_{18}N_2O$	219	84, 77, 67, 55	10
Tapentadol HCl	175591-09-0	$C_{14}H_{23}NO$	222	135, 121, 107, 77	10
Norfentanyl	1609-66-1	$C_{14}H_{20}N_2O$	233	146, 94, 84, 55	10
Isobutyryl norfentanyl	1046436-53-6	$C_{15}H_{22}N_2O$	247	177, 146, 84, 55	5
Butyryl norfentanyl HCl	N/A	$C_{15}H_{22}N_2O$	247	177, 146, 84, 55	50
N-methyl norfentanyl HCl	24775-71-1	$C_{15}H_{22}N_2O$	247	98, 94, 70, 55	5
(±)-trans-3-methyl norfentanyl	33794-43-3	$C_{15}H_{22}N_2O$	247	150, 98, 69, 56	10
(±)-cis-3-methyl Norfentanyl	33794-42-2	$C_{15}H_{22}N_2O$	247	150, 98, 69, 56	10
Furanyl norfentanyl HCl	N/A	$C_{16}H_{18}N_2O_2$	271	188, 95, 84, 55	40
4-ANPP (N-Phenyl-1-(2-phenylethyl)-4-piperidinamine)	21409-26-7	$C_{19}H_{24}N_2$	281	188, 105, 79	20
Despropionyl *m*-fluorofentanyl	416881-38-4	$C_{19}H_{23}FN_2$	299	188, 105, 79	5
Despropionyl *o*-fluorofentanyl	864422-91-3	$C_{19}H_{23}FN_2$	299	188, 105, 79	5
Despropionyl *p*-fluorofentanyl	122861-41-0	$C_{19}H_{23}FN_2$	299	188, 105, 79	5
(±)-Methadone HCl	1095-90-5	$C_{21}H_{27}NO$	310	265, 117, 105, 91, 77, 57	10
Benzyl acrylfentanyl HCl	N/A	$C_{21}H_{24}N_2O$	321	174, 91	5
Benzyl fentanyl HCl	5156-58-1	$C_{21}H_{26}N_2O$	323	174, 91	5
Acetyl fentanyl HCl	117332-89-5	$C_{21}H_{26}N_2O$	323	188, 105, 79	5
Furanylethyl fentanyl HCl	1443-49-8	$C_{20}H_{26}N_2O_2$	327	245, 178, 176, 146, 95, 84, 67	10
6-monoacetylmorphine	2784-73-8	$C_{19}H_{21}NO_4$	328	211, 193, 181, 165	10
U-47700	82657-23-6	$C_{16}H_{22}Cl_2N_2O$	329	33, 286, 284, 204, 172, 157, 144	5
Thienyl fentanyl HCl	117332-93-1	$C_{19}H_{24}N_2OS$	329	180, 97, 82	10
Acrylfentanyl HCl	79279-03-1	$C_{22}H_{26}N_2O$	335	188, 105, 79	5
Fentanyl HCl	1443-54-5	$C_{22}H_{28}N_2O$	337	188, 105, 79	5
α-Methyl acetyl fentanyl HCl	N/A	$C_{22}H_{28}N_2O$	337	202, 119, 91, 84	5
4'-Methyl acetyl fentanyl HCl	1071703-95-1	$C_{22}H_{28}N_2O$	337	202, 119, 91	10
Papaverine	58-74-2	$C_{20}H_{21}NO_4$	340	324, 296, 202, 171	20
6-Acetylcodeine	6703-27-1	$C_{20}H_{23}NO_4$	342	282, 225, 165	10
U-51754 HCl	N/A	$C_{17}H_{24}Cl_2N_2O$	343	300, 298, 218, 157, 112, 81, 79	5

U-48800 HCl	N/A	$C_{17}H_{24}Cl_2N_2O$	343	300, 298, 218, 159, 112, 81, 79, 70	5
Thiofentanyl HCl	79278-88-9	$C_{20}H_{26}N_2OS$	343	245, 194, 146, 111, 84, 77	5
Cyclopropyl fentanyl HCl	N/A	$C_{23}H_{28}N_2O$	349	188, 105, 79, 68	5
4'-Methyl fentanyl HCl	1071703-97-3	$C_{23}H_{30}N_2O$	351	202, 119, 91	5
α-Methyl fentanyl HCl	1443-44-3	$C_{23}H_{30}N_2O$	351	202, 119, 91	5
β-Methylfentanyl HCl	1443-43-2	$C_{23}H_{30}N_2O$	351	188, 146, 134, 105	5
Butyryl fentanyl HCl	1443-52-3	$C_{23}H_{30}N_2O$	351	188, 105, 79	5
Isobutyryl fentanyl HCl	117332-90-8	$C_{23}H_{30}N_2O$	351	188, 105, 79	5
(±)-cis-3-Methyl fentanyl HCl	78995-18-3	$C_{23}H_{30}N_2O$	351	202, 134, 105	5
(±)-trans-3-Methyl fentanyl HCl	78995-09-2	$C_{23}H_{30}N_2O$	351	202, 134, 105	5
m-Methylfentanyl HCl	1465-22-1	$C_{23}H_{30}N_2O$	351	188, 146, 134, 105	5
o-Methylfentanyl HCl	1443-53-4	$C_{23}H_{30}N_2O$	351	188, 146, 134, 105	5
p-Methylfentanyl HCl	1807-12-1	$C_{23}H_{30}N_2O$	351	188, 146, 134, 105	5
o-Fluoro acrylfentanyl HCl	N/A	$C_{22}H_{25}FN_2O$	353	188, 105, 79	10
p-Fluoro acrylfentanyl	N/A	$C_{22}H_{25}FN_2O$	353	188, 105, 79	5
β-Hydroxy fentanyl HCl	1473-95-6	$C_{22}H_{28}N_2O_2$	353	335, 204, 202, 186, 174, 132, 91	10
Methoxyacetyl fentanyl HCl	101365-54-2	$C_{22}H_{28}N_2O_2$	353	188, 105, 79	10
m-Fluorofentanyl HCl	N/A	$C_{22}H_{27}FN_2O$	355	188, 105	5
o-Fluorofentanyl HCl	N/A	$C_{22}H_{27}FN_2O$	355	188, 105	5
p-Fluorofentanyl HCl	117332-92-0	$C_{22}H_{27}FN_2O$	355	188, 105	5
3-Fluorofentanyl HCl	N/A	$C_{22}H_{27}FN_2O$	355	188, 105	10
α-Methyl thiofentanyl HCl	117332-94-2	$C_{21}H_{28}N_2OS$	357	259, 208, 125, 97	5
U-69593	96744-75-1	$C_{22}H_{32}N_2O_2$	357	286, 168, 150, 137, 109, 95, 91, 71	5
β-Hydroxythiofentanyl HCl	N/A	$C_{20}H_{26}N_2O_2S$	359	341, 192, 146, 132, 111, 97	20
Cyclobutyl fentanyl HCl	N/A	$C_{24}H_{30}N_2O$	363	188,105, 55	5
Valeryl fentanyl HCl	117332-91-9	$C_{24}H_{32}N_2O$	365	188, 105, 79	5
(±)-cis-3-methyl butyryl fentanyl HCl	88641-20-7	$C_{24}H_{32}N_2O$	365	202, 134, 105, 79, 69	5

α-methyl butyryl fentanyl HCl	N/A	$C_{24}H_{32}N_2O$	365	188, 105, 57	5
p-Fluoro cyclopropyl fentanyl HCl	N/A	$C_{23}H_{27}FN_2O$	367	188, 134, 105, 69	5
p-Methoxyfentanyl HCl	23609-41-8	$C_{23}H_{30}N_2O_2$	367	188, 134, 105	5
m-Fluoroisobutyryl fentanyl HCl	N/A	$C_{23}H_{29}FN_2O$	369	188, 105, 79	5
o-Fluoroisobutyryl fentanyl HCl	N/A	$C_{23}H_{29}FN_2O$	369	188, 105, 79	5
p-Fluoroisobutyryl fentanyl HCl	2309383-06-8	$C_{23}H_{29}FN_2O$	369	188, 105, 79	5
m-Fluorobutyryl fentanyl HCl	N/A	$C_{23}H_{29}FN_2O$	369	188, 105, 79	5
o-Fluorobutyryl fentanyl HCl	N/A	$C_{23}H_{29}FN_2O$	369	188, 105	5
p-Fluorobutyryl fentanyl HCl	N/A	$C_{23}H_{29}FN_2O$	369	188, 105	5
Heroin	561-27-3	$C_{21}H_{23}NO_5$	370	328, 310, 268, 165, 58	30
p-Chlorofentanyl HCl	117994-27-1	$C_{22}H_{27}ClN_2O$	371	188, 134, 105	10
m-Fluoro methoxyacetyl fentanyl HCl	N/A	$C_{22}H_{27}FN_2O_2$	371	188, 105	5
p-Fluoro methoxyacetyl fentanyl HCl	N/A	$C_{22}H_{27}FN_2O_2$	371	188, 105	5
N-Benzyl phenyl norfentanyl	N/A	$C_{25}H_{26}N_2O$	371	188, 174, 170, 105, 91, 77	5
Furanyl fentanyl HCl	101365-56-4	$C_{24}H_{26}N_2O_2$	375	188, 105	5
Furanyl fentanyl 3-furancarboxamide isomer HCl	N/A	$C_{24}H_{26}N_2O_2$	375	188, 105, 95	5
Cyclopentyl fentanyl HCl	N/A	$C_{25}H_{32}N_2O$	377	188, 105, 69	5
Tetrahydrofuran fentanyl HCl	N/A	$C_{24}H_{30}N_2O_2$	379	188, 105, 71	5
Benzyl carfentanil HCl	N/A	$C_{23}H_{28}N_2O_3$	381	349, 321, 232, 146, 113, 91	10
p-methoxy butyryl fentanyl HCl	N/A	$C_{24}H_{32}N_2O_2$	381	188, 124, 105	5
p-Chloroisobutyryl fentanyl HCl	N/A	$C_{23}H_{29}ClN_2O$	385	188, 134, 105	5
Phenyl fentanyl HCl	N/A	$C_{26}H_{28}N_2O$	385	188, 105, 77	5
Sufentanil Citrate	60561-17-3	$C_{22}H_{30}N_2O_2S$	387	238, 206, 140, 111, 99, 77, 67	5
Cyclohexyl fentanyl HCl	N/A	$C_{26}H_{34}N_2O$	391	188, 105, 83, 55	5
Carfentanil	59708-52-0	$C_{24}H_{30}N_2O_3$	395	363, 335, 246, 134, 113, 105, 81	5
p-Fluoro tetrahydrofuran fentanyl HCl	N/A	$C_{24}H_{29}FN_2O_2$	397	188, 105, 71	10
2,2,3,3-Tetramethyl-cyclopropyl fentanyl HCl	N/A	$C_{27}H_{36}N_2O$	405	281, 186, 125, 105, 97, 55	5
Noscapine	128-62-1	$C_{22}H_{23}NO_7$	414	353, 220	60

Benzodioxole fentanyl	N/A	$C_{27}H_{28}N_2O_3$	429	188, 149, 121, 105	5
Buprenorphine HCl	53152-21-9	$C_{29}H_{41}NO_4$	468	418, 149, 84, 79, 69, 55	30

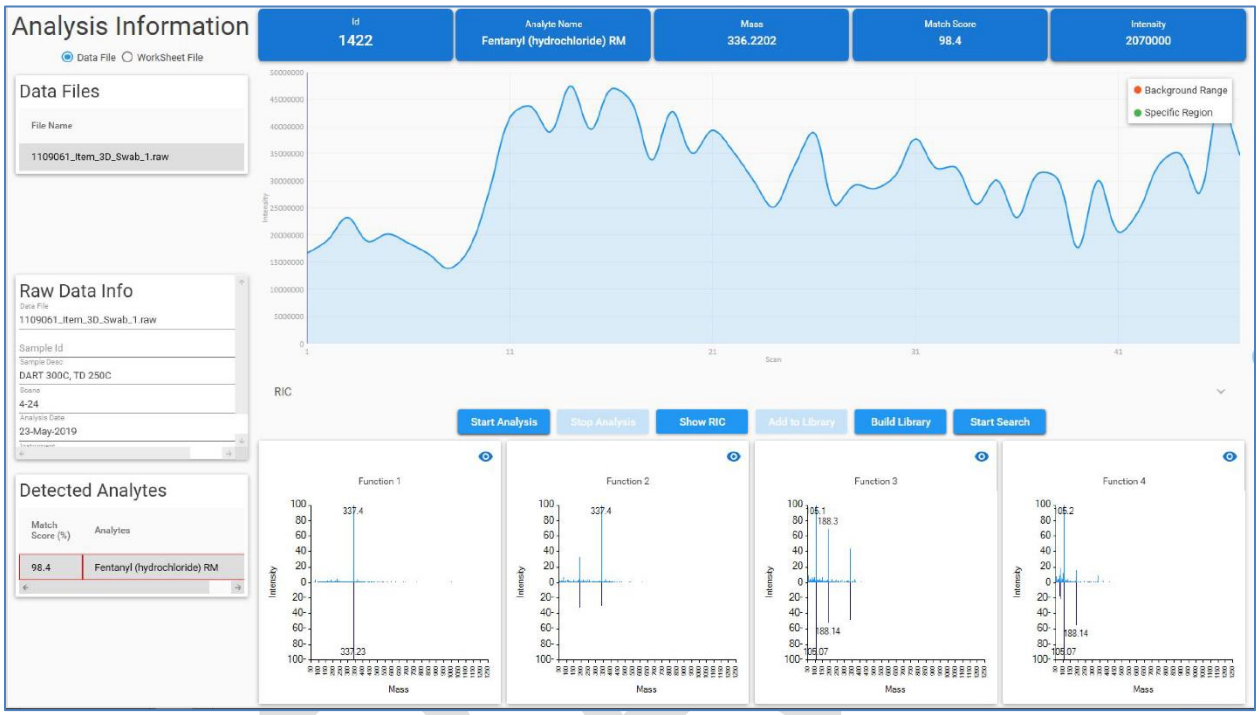

Figure 2: Example of a database match to fentanyl, displaying compound name, mass, total ion chronogram, algorithm match score of the sample spectra to the database entry, and the sample mass spectra collected at four different collision energies (15, 30, 50, and 70 V) (top spectrum of each Function) compared to the ions matched to the database entry (bottom spectrum of each Function).

Results and Discussion

Table 1 lists the opioid standards analyzed and their minimum detectable levels for the DART-MS method. The NextGenPIMISA® software uses a proprietary algorithm to match the sample spectra collected at four different cone voltages to reference spectra in the database collected under the same conditions. Many of the fentanyl analogs are structural isomers with several common fragments ions, which can result in multiple database matches or false positives. For example, isobutyryl norfentanyl and butyryl norfentanyl cannot be differentiated, nor can the other isomers with similar fragment ions. However, there were no observed "true" false positives, i.e. for every standard listed below the correct compound was detected 100% of the time, although additional matches were often observed. Figure 2 shows an example of a database match to fentanyl.

The newspaper, disposable wipes, face masks, plastic bags, and bubble wrap

with duct tape were challenging samples for techniques requiring extractions, such as LC-MS and GC-MS, but well suited for analysis using the DART-MS. Swabs were simply rubbed across the stained portions of the items and then examined. Table 2 compares the results from the three techniques. Analysis using LC-MS detected a multitude of compounds including fentanyl, fentanyl analogs, heroin, and opioid alkaloids, all of which were confirmed by comparison of retention time and mass spectral profiles to those of reference standards. Analysis using GC-MS with reference standards detected fewer analytes and the DART-MS analysis with database matching identified even less in all cases excluding Item 6 (bubble wrap and duct tape). This is not unexpected for the DART-MS results, considering the lack of chromatographic separation and the non-homogeneity of the samples. The LC-MS and GC-MS experiments were conducted on solvent extracts while the DART-MS experiments were conducted on swabs rubbed on the visibly contaminated areas of the Items which may account for the variability in the results. The non-porous surfaces of Item 6 may have made it more amendable to a swabbing technique versus an extraction. However, for each sample at least one common compound (typically fentanyl) was detected by all three techniques, indicating that analysis using the DART-MS would be a suitable technique for screening samples in a non-laboratory field setting as a rapid "pass/fail" test for a variety of analytes, including dangerous and illicit drugs such as opioids.

Table 2. Comparison of results using DART-MS, LC-MS, and GC-MS. Compounds listed in bold were supported by DART-MS.

Sample	**DART-MS**	**LC-MS**	**GC-MS**
Item 2 (newspaper)	**Fentanyl, noscapine**	**Fentanyl**, **noscapine**, heroin, valeryl fentanyl, papaverine, 6-acetylcodeine, 6-monoacetylmorphine, acetyl fentanyl, 4-ANPP	**Fentanyl**, heroin, valeryl fentanyl, 6-acetylcodeine, 6-monoacetylmorphine
Item 3A (disposable wipes)	**Fentanyl,** methoxy acrylfentanyl	**Fentanyl**, heroin, valeryl fentanyl, noscapine, 6-acetylcodeine, 6-monoacetylmorphine, acetyl fentanyl, 4-ANPP, papaverine,	**Fentanyl**, heroin, valeryl fentanyl, noscapine, 6-acetylcodeine, 6-monoacetylmorphine
Item 3B (disposable wipes)	**Fentanyl,** β-methyl acetyl fentanyl, α-methyl acetyl fentanyl	**Fentanyl,** heroin, valeryl fentanyl, noscapine, papaverine, 6-acetylcodeine, 6-monoacetylmorphine	**Fentanyl**

Item 3C (disposable wipes)	**Fentanyl, noscapine,** valeryl fentanyl, methoxy acrylfentanyl, methyl isobutyryl fentanyl	**Fentanyl, noscapine,** valeryl fentanyl, heroin, papaverine, 6-acetylcodeine, 6-monoacetylmorphine	**Fentanyl**, heroin, **noscapine**
Item 3D (disposable wipes)	**Fentanyl**	**Fentanyl**, heroin, valeryl fentanyl, 6-acetylcodeine, 6-monoacetylmorphine, noscapine, papaverine,	**Fentanyl**, heroin, valeryl fentanyl, 6-acetylcodeine, 6-monoacetylmorphine
Item 4A (face masks)	**Fentanyl**	**Fentanyl,** heroin, 6-monoacetylmorphine valeryl fentanyl, noscapine, papaverine, 6-acetylcodeine	**Fentanyl,** heroin, 6-monoacetylmorphine, caffeine
Item 5D (plastic bags)	**Fentanyl,** β-methyl fentanyl, α-methyl fentanyl	**Fentanyl,** heroin, valeryl fentanyl, noscapine, papaverine, 6-acetylcodeine, 6-monoacetylmorphine, acetyl fentanyl	**Fentanyl,** heroin, 6-acetylcodeine
Item 6 (bubble wrap and duct tape)	**Fentanyl, heroin**, **6-monoacetylmorphine**, β-methyl acetyl fentanyl, α-methyl **acetyl fentanyl, methamphetamine,** 4-methyl acetyl fentanyl, phentermine, 3,4-methylenedioxy-N-benzylcathinone	**Fentanyl, heroin**, **6-monoacetylmorphine,** noscapine, papaverine, 6-acetylcodeine**, acetyl fentanyl, methamphetamine**	**Heroin**

While the DART-MS screening technique does not provide as much complete information as analysis using LC-MS or GC-MS, it does have several advantages that make it a useful field-deployable tool. Minimal sample preparation is required, eliminating the need for solvents and supplies such as vials, filters, and pipettes. The sampling and data acquisition time of the DART-MS is 20 seconds and data processing requires an additional 30-40 seconds for each sample, allowing for rapid identification, which is particularly useful for dangerous samples. In comparison, run times for the LC-MS and GC-MS methods range from 10 – 30 minutes per sample, not including the time required for sample preparation. Eliminating the need to extract, filter, and dilute the samples provides additional time savings and requires less supplies. The DART-MS instrument has a larger footprint than hand-held devices, but it is smaller than traditional benchtop mass spectrometers. The power requirements are readily met, the startup time for the mass analyzer is about 10 minutes to reach the required vacuum, and the maintenance is also minimal – calibration is only required annually. If the sample contains an active ingredient that is not present in the database, the mass spectra can

be interrogated manually, and the CID fragmentation information gives the analyst greater confidence in their findings. Additionally, the user can add new entries to the library easily, provided a standard is available.

Conclusion

A rapid screening technique has been developed to analyze 87 standards of opioids and drugs of abuse and the minimum detectable levels observed indicate that this method will have sufficient sensitivity for analyzing finished dosage forms including tablets, capsule contents, and injectables, as well as bulk powders and liquids. This method was used to analyze a sample sent to the FDA's Forensic Chemistry Center consisting of newspaper, disposable wipes, face masks, plastic bags, and bubble wrap with duct tape that were all contaminated with illicit drugs and the results were compared to those collected using both LC-MS and GC-MS. The overall results for the screening technique were not as comprehensive as those observed from the other methods, but at least one common compound (typically fentanyl) was detected by all three techniques for each sample, indicating that the DART-MS screening method can provide useful information rapidly with minimal sample preparation. Portable orthogonal techniques such as Raman or Fourier Transform Infrared (FT-IR) spectroscopy could also be utilized in the field to provide greater confidence in the database matches, particularly when multiple analytes with the same monoisotopic mass are detected.

Acknowledgement/Disclaimer

The opinions expressed in this manuscript are those of the authors, and do not necessarily reflect the current or future official policy or opinion of the U.S. Food & Drug Administration. The mention of trade name or specific commercial products is for clarification of the methods and should not be considered endorsement by the U.S. Food & Drug Administration. The authors would like to thank Frederick Li of IonSense for many helpful discussions and guidance.

References

[1] "Drug Overdoes Deaths in the U.S. Top 100,000 Annually", National Center for Health Statistics. November 17, 2021. Drug Overdose Deaths in the U.S. Top 100,000 Annually (cdc.gov)

[2] Cooman T., Ott C.E., Dalzell K.A., Burns A., Sisco E., Arroyo L.E. (2021). Screening of seized drugs utilizing portable Raman spectroscopy and Direct Analysis in Real Time-Mass Spectrometry (DART-MS). *Forensic Chemistry*. https://doi.org/10.1016/j.forc.2021.100352

[3] de O. Penido C.A.F., Pacheco, M.T.T., Lednev I.K., Silveira L., (2016) Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse, *Journal of Raman Spectroscopy*, *47* 28–38. http://dx.doi.org/10.1002/jrs.4864

[4] Drug Enforcement Administration, U.S. Department of Justice, DEA-DCT-DIB-021-16, Counterfeit

Prescription Pills Containing Fentanyls: A Global Threat 2. (2016).

[5] FDA's Budget: Advancing the Goal of Ending the Opioid Crisis. FDA's Budget: Advancing the Goal of Ending the Opioid Crisis | FDA, (accessed August 27, 2021).

[6] Lanzarotta A., Kern S., Batson J., Falconer T.M., Fulcher M., Gaston K.W., Kimani M.M., Lorenz L., Morales-Garcia F., Ranieri N., Skelton D., Thatcher M.D., Toomey V.M., Voelker S., Witkowski M.R. (2021) Evaluation of "Toolkit" consisting of handheld and portable analytical devices for detecting active pharmaceutical ingredients in drug products collected during a simultaneous nation-wide mail blitz. *Journal of Pharmaceutical and Biomedical Analysis, 203* 114183. https://doi.org/10.1016/j.forc.2020.100294

[7] Lanzarotta A., Witkowski M., Batson J. (2020) Identification of Opioids and Related Substances using Handheld Raman Spectrometers. *Forensic Science, 65*(2) 421-427, http://dx.doi: 10.1111/1556-4029.14217

[8] Mainali D., Seelenbinder J. *(*2016) Automated fast screening method for cocaine identification in seized drug samples using a portable Fourier transform infrared (FT-IR) instrument. *Applied Spectroscopy, 70* 916–22. https://doi: 10.1177/0003702816638305.

[9] National Drug Threat Assessment, Drug Enforcement Agency, (2018). 2018 National Drug Threat Assessment (NDTA) (dea.gov)

[10] Sisco E. and Forbes T.P. (2021) Forensic applications of DART-MS: A review of recent literature. *Forensic Chemistry, 22* 100294. https://doi.org/10.1016/j.forc.2020.100294

[11] Sisco E., Forbes T.P., Staymates M.E., Gillen G. (2016) Rapid analysis of trace drugs and metabolites using a thermal desorption DART-MS configuration. *Analytical Methods, 8* 6494-6499. http://dx.doi.org/10.1039/C6AY01851C.

[12] Sisco E., Robinson E., Burns A., Mead R. (2019) What's in the bag? Analysis of exterior drug packaging by TD-DART-MS to predict the contents. *Forensic Science International, 304* 1-7. http://dx.doi.org/10.1016/j.forsciint.2019.109939

[13] Sisco E., Verkouteren J., Staymates J., Lawrence J. (2017) Rapid detection of fentanyl, fentanyl analogues, and opioids for an on-site or laboratory based drug seizure screening using thermal desorption DART-MS and ion mobility spectrometry. *Forensic Chemistry 4* 108-115. http://dx.doi.org/10.1016/j.forc.2017.04.001.

[14] Sorak D., Herberholz L., Iwascek S., Altinpinar S., Pfeifer F., Siesler H.W. (2012) New developments and applications of handheld Raman, mid-infrared, and near-infrared spectrometers. *Applied Spectroscopy Reviews, 47* 83–115. https://doi.org/10.1080/05704928.2011.625748

[15] Wide-ranging online data for epidemiologic research (WONDER). Atlanta, GA: CDC, National Center for Health Statistics; 2020. Available at http://wonder.cdc.gov.

[16] Zaknoun, H., Binette M.-J., Tam M. (2019) Analyzing fentanyl and fentanyl analogues by ion mobility spectrometry. *International Journal of Ion Mobility Spectrometry, 22* 1–10. http://dx.doi.org/10.1007/s12127-019-00244-0.