Surveillance of a Multiple Opioids in API's and Drug Products Using High Performance Liquid Chromatography High Resolution Mass Spectrometry

Geerlof-Vidavsky, Ilan[1]; Stark, Matthew[1]; Balch, Maurie[1]; Ely, Tamara[2]; Godwin, Francis[2]; Ruzicka, Connie[1]
[1]FDA/CDER/OPQ/OTR, [2]FDA/CDER/OC/OMQ

Abstract

Background: In recent years, two-thirds of drug overdose deaths involved opioid-related products, primarily by misuse of synthetic opioids, like fentanyl. In response to the opioid crisis, the FDA is focusing efforts to reduce the impact of opioid abuse on American communities through the "Opioid Action Plan". One component of this plan is to survey the integrity of Active Pharmaceutical Ingredients (APIs) and finished drug products in the commercially marketed US supply chain. Surveillance allows the agency to assess product quality and monitor for adulteration of opioid products, thus reducing the probability of an economically-motivated adulterated product making it to market.

Purpose: To develop and validate a multi-analyte opioid High-Performance Liquid Chromatography High Resolution Mass Spectrometry (HPLC-HRMS) method for the quality assessment of opioid drug products. Use of a multi-analyte method allows for sample versatility and improved efficiency; other higher-throughput methods are often compound specific and not designed to accommodate a variety of impurities or matrices.

Methodology: A HPLC-HRMS method was developed and validated for the detection of 24 opioid compounds, and the method analysis time was 34 minutes. The method was used for identification of the label claim ingredient, screening for economically motivated adulterants, impurity quantitation and assay in APIs. Adapting the method for analysis of finished products expanded the compound list to 23 opioids, 2 agonists and 2 antagonists reduced analysis time to 16 minutes. The average LOD/LOQ, in full MS mode, were 0.19/0.56 ng/mL.

Results: Ninety-three API samples were evaluated. All samples met specifications in assay (NLT 98%, NMT 102%) and individual opioid impurity limits. Finished drug products were tested for assay only and all 64 samples evaluated met specifications (NLT 90%, NMT 110%). Additionally, no economically motivated adulteration was detected in either API or finished drug product samples.

Conclusions: A multi-analyte opioid HPLC-HRMS method was developed, validated and implemented for the quality assessment of opioid API and finished drug products. All samples met specifications and no economically motivated adulteration was detected. Results from this study provide increased confidence in the commercially marketed opioid supply chain. Future phases of this work will focus on imported and compounded opioid drug products.

Introduction

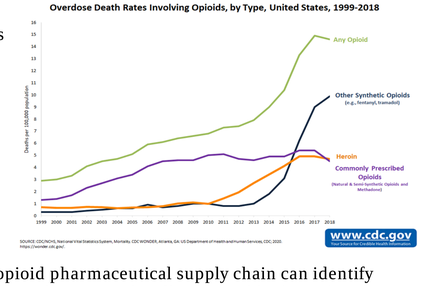
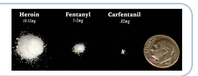


Figure 1. Death rates attributed to opioid overdoses have increased in recent years and a sharp increase has been observed for use of synthetic opioids.

- Surveillance of the opioid pharmaceutical supply chain can identify potential quality risks and ensure safe, effective and high-quality drug products are available.
- Economically motivated adulteration detected in the illegal drug supply chain
  - *Heroin laced with fentanyl*
  - *Fentanyl laced counterfeit drugs*

Lethal Doses (Dime for Scale)



- Many USP monographs for opioids lack specificity and are outdated
- Use of compound/product specific methods can be cumbersome, time consuming and may lack ability to detect opioid substitution

Materials and Methods

Solution Preparations:

- Stock standards and samples were prepared in 50% methanol to a concentration of ~1 mg/mL.
- Stock solutions were diluted to 0.1-1.0 µg/mL in mobile phase A
- A 1 µg/mL deuterated analog of the standard was also included

Instrument: Thermo Q-Exactive HF or HF-X series HPLC/HRMS	
HPLC Parameters	
Mobile Phase:	Gradient Elution
A	2.5 mM Ammonium Formate, pH 3.8
B	Acetonitrile
Column:	Waters Acquity BEH C18 ,1.7 µm, 2.1 x 150 mm
Flow Rate:	0.3 mL/min
Column Temperature:	30 °C
Mass Spectrometry Parameters	
Resolving Power:	60,000
Mode:	Positive
Scan Range:	145-800 m/z
MS Mode:	Used for quantitation
MS/MS Mode:	Used for identification (See Table 3)
Selection Window:	1.5 m/z
Resolving Power:	30,000

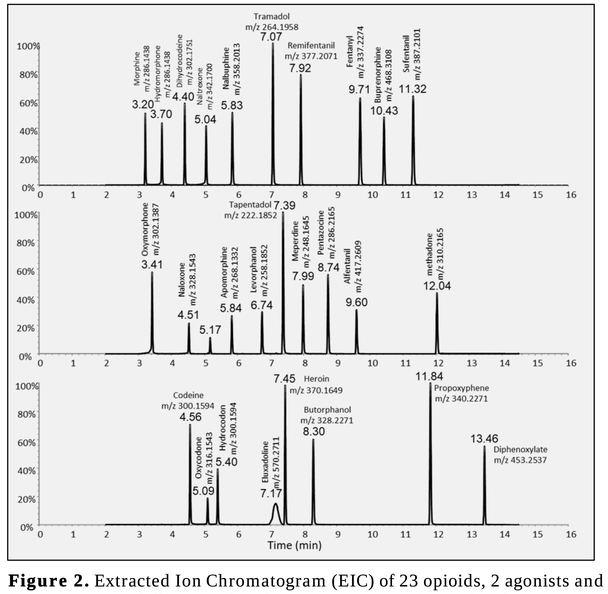


Figure 2. Extracted Ion Chromatogram (EIC) of 23 opioids, 2 agonists and 2 antagonists in full MS mode. Chromatogram is in 3 axes for clarity.

Results and Discussion

- Figure 2 shows the exact mass EIC for a 1 µL injection of an opioid standard containing 27 compounds at concentrations of ~1 µg/mL detected in the full MS mode. All 27 compounds are well separated within 16 minutes.
- Table 3 provides a list of opioid compounds included in the method, their molecular weight, retention time, recovery at the lowest concentration, limit of detection, mass of fragment ions and relative energy.
- Positive identification of opioids in a sample is confirmed by retention time, exact mass, and two fragment ions.
- Quantification is normalized to the internal standard of the deuterated API in the buffer.
- Ninety-three API samples were collected from the commercially marketed US supply chain and evaluated for assay and impurities using the HPLC-HRMS method.
- All samples met specifications in identification, assay (NLT 98%, NMT 102%) and individual opioid impurity limits. A summary of results are presented in Table 1.

Table 1. Collected API samples statistics and results, all samples passed specifications

API	Samples	Manufacturers*	Dealers*	Pass
Buprenorphine HCl	20	10	10	yes
Fentanyl Citrate	14	6	7	yes
Hydrocodone HCl	11	5	7	yes
Hydromorphone	13	5	5	yes
Morphine Sulfate	13	5	8	yes
Nalbuphine HCl	6	2	4	yes
Sufentanil Citrate	7	2	5	yes
Tramadol HCl	9	3	7	yes
Total	93			

*Multiple lots were evaluated for several manufactures

- Sixty-four opioid-containing finished drug products were tested and found to meet specifications in identification and assay (NLT 90%, NMT 110%). A summary of results are presented in Table 2.
- No economically motivated adulteration were detected in either API or finished drug products samples.

Table 2. Collected DP samples statistics and results, all samples passed specifications

Drug Products	Samples	Manufacturers*	Dealers*	Pass
Buprenorphine	2	2	2	yes
Buprenorphine/Naloxone	6	5	2	yes
Fentanyl	6	2	2	yes
Hydrocodone/Acetaminophen	15	10	6	yes
Methadone	4	4	3	yes
Morphine	1	1	1	yes
Naloxone	1	1	1	yes
Naltrexone	4	2	1	yes
Opium tincture	2	1	1	yes
Oxycodone	11	8	6	yes
Oxycodone/Acetaminophen	11	6	2	yes
Tramadol	1	1	1	yes
Total	64			

*Multiple lots were evaluated from several manufactures

Table 3. List of opioid compounds, validation and experimental details. Recoveries were calculated using quadratic calibration. MS/MS product-ion spectra were used for positive identification of opioids.

Compound (Acronym)	Composition	m/z	Rt min	Recovery at 0.05 µg/mL	LOD ng/mL	NCE %	MS/MS fragments
Morphine Sulfate (MOR)	$C_{17}H_{19}NO_3$	286.1438	3.20	98.7%	0.05	60	201.0910, 58.0651
Oxymorphone (OM)	$C_{17}H_{19}NO_4$	302.1387	3.41	100.8%	0.18	50	284.1281, 227.0941
Hydromorphone HCl (HM)	$C_{17}H_{19}NO_3$	286.1438	3.70	101.8%	0.22	60	227.0703, 185.0597
Dihydrocodeine Bitartrate (DH)	$C_{18}H_{23}NO_3$	302.1751	4.40	100.9%	0.12	60	245.1172, 199.0754
Naloxone* (NLX)	$C_{19}H_{21}NO_4$	328.1543	4.51	103.4%	0.19	45	268.1332, 253.1097
Codeine Sulfate (COD)	$C_{18}H_{21}NO_3$	300.1594	4.56	99.5%	0.09	60	215.1067, 183.0804
Naltrexone* (NTX)	$C_{20}H_{23}NO_4$	342.1700	5.04	103.8%	0.08	45	282.1489, 270.1125
Oxycodone (OC)	$C_{18}H_{21}NO_4$	316.1543	5.09	106.2%	0.27	60	256.1332, 241.1097
Hydrocodone bitartrate (HC)	$C_{18}H_{21}NO_3$	300.1594	5.40	99.9%	0.26	60	241.0859, 199.0754
Nalbuphine HCL (NAL)	$C_{21}H_{27}NO_4$	358.2013	5.83	105.5%	0.11	50	340.1907, 296.1645
Apomorphine HCl (AM)	$C_{17}H_{17}NO_2$	268.1332	5.84	105.1%	0.24	50	237.0910, 191.0855
Levorphanol Tartrate (LVP)	$C_{17}H_{23}NO$	258.1852	6.74	103.9%	0.13	60	199.1117, 133.0648
Tramadol HCl (TRA)	$C_{16}H_{25}NO_2$	264.1958	7.07	103.3%	0.12	20	246.1852, 58.0651
Eluxadoline (ED)	$C_{32}H_{35}N_5O_5$	570.2711	7.17	100.0%	1.05	30	401.1694, 171.0917
Tapentadol HCl (TAD)	$C_{14}H_{23}NO$	222.1852	7.39	102.2%	0.11	50	135.0804, 107.0491
Heroin (HER)	$C_{21}H_{23}NO_5$	370.1649	7.45	103.6%	0.24	70	181.0648, 165.0699
Remifentanil HCl (REM)	$C_{20}H_{28}N_2O_5$	377.2071	7.92	101.2%	0.07	50	317.1860, 228.1230
Meperidine HCl (MEP)	$C_{15}H_{21}NO_2$	248.1645	7.99	103.5%	0.07	60	220.1332, 174.1277
Butorphanol Tartrate (BTP)	$C_{21}H_{29}NO_2$	328.2271	8.30	98.7%	0.09	60	310.2165, 185.0961
Pentazocine (PEN)	$C_{19}H_{27}NO$	286.2165	8.74	99.8%	0.07	40	218.1539, 69.0699
Alfentanil HCl (ALF)	$C_{21}H_{32}N_6O_3$	417.2609	9.60	105.3%	0.11	30	385.2347, 268.1768
Fentanyl Citrate (FEN)	$C_{22}H_{28}N_2O$	337.2274	9.71	102.5%	0.12	40	188.1434, 105.0699
Buprenorphine HCl** (BUP)	$C_{29}H_{41}NO_4$	468.3108	10.43	100.4%	0.15	60	396.2169, 187.0754
Sufentanil Citrate (SUF)	$C_{22}H_{30}N_2O_5S$	387.2101	11.32	101.3%	0.17	30	355.1839, 238.1260
(+)-Propoxyphene HCl (PRP)	$C_{22}H_{29}NO_2$	340.2271	11.84	101.3%	0.13	20	266.1903, 58.0651
Methadone HCl** (MET)	$C_{21}H_{27}NO$	310.2165	12.04	100.7%	0.22	30	265.1587, 105.0335
Diphenoxylate HCl (DFL)	$C_{30}H_{32}N_2O_2$	453.2537	13.46	103.4%	0.34	50	425.2224, 187.0992

*Opioid antagonist, **opioid agonist

Conclusions

- A multi-analyte opioid HPLC-HRMS method was developed, validated and implemented for the quality assessment of opioid API and finished drug products.
- Method validation included determination of LOD, LOQ, linearity, precision and recovery. System suitability parameters were established.
- MS/MS detection is used for identification. If an interference is observed, an exact MS/MS quantitation method will be revisited.
- 93 API samples and 64 drug product sample from the commercially marketed US supply chain were evaluated.
- No evidence of economically motivated adulteration was detected, and all samples evaluated met quality specifications.
- Results from this study provide increased confidence in the legal opioid supply chain and demonstrate that safe, effective high-quality opioid products are available.
- Future phases of this work are planned and will focus on imported and compounded opioid drug products.