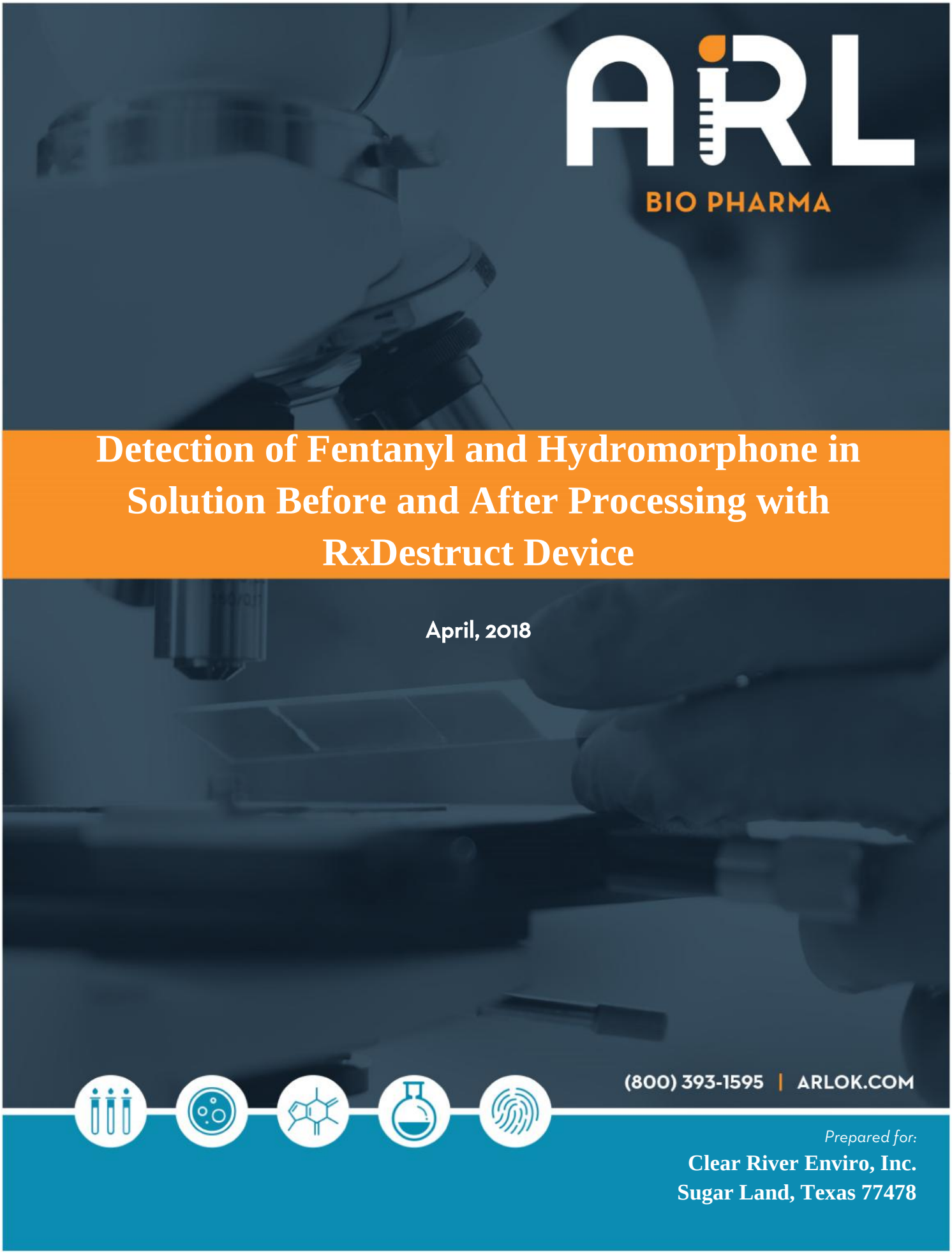

Detection of Fentanyl and Hydromorphone in Solution Before and After Processing with RxDestruct Device

April, 2018

(800) 393-1595 | ARLOK.COM

Prepared for:

Clear River Enviro, Inc.
Sugar Land, Texas 77478

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TEST SITE

ARL BioPharma, Inc.
840 Research Parkway, Suite 546
Oklahoma City, OK 73104

STUDY SPONSOR

Clear River Enviro, Inc.
106 Capri Street
Sugar Land, Texas 77478

SIGNATURES

The reported methods and procedures contained in this report were found to describe those used, and the results to constitute an accurate and complete reflection of the study raw data. The effective date of the report is defined as the date of the latest signature

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OVERVIEW

Clear River Enviro, Inc. would like to demonstrate the efficiency of their RxDestruct Device. The Rx Destruct process is based on Fenton's reaction. Fenton's reagent (a combination of Hydrogen Peroxide and Ferrous Iron) produces strong oxidizing species capable of degrading drug substances. Two solutions each of Fentanyl and Hydromorphone HCl, at typical therapeutic concentrations, were exposed to the Rx Destruct process. Post-destruct samples were analyzed for traces of Fentanyl and Hydromorphone HCl.

The purpose of the project described herein was to develop a LC-MS/MS method for the detection of Fentanyl and Hydromorphone in solutions after processing in the RxDestruct device. The method was then used to assess the efficiency of the destruction of these drug substances by the RxDestruct device. In this study, two solutions of Fentanyl (1 mcg/mL and 1.5-2 mcg/mL) in Normal Saline, and two solutions of Hydromorphone HCl (8 mcg/mL and 12 mcg/mL) in Normal Saline, were exposed to Fenton's reagent through the RxDestruct device.

Results indicate that there was significant destruction of both Fentanyl and Hydromorphone after processing in the RxDestruct device. Large peaks for both Fentanyl and Hydromorphone HCl were detected in respective pre-destruct samples. In post-destruct samples there was either no peak detected, or the level was below that of the established Limit of Quantitation for this method. A summary of results for both pre-destruction and post-destruction samples can be found in Tables 1 and 2. See also Figures 1 through 4 for representative chromatograms.

Figure 1: 1 mcg/mL Fentanyl Pre-destruct Chromatogram

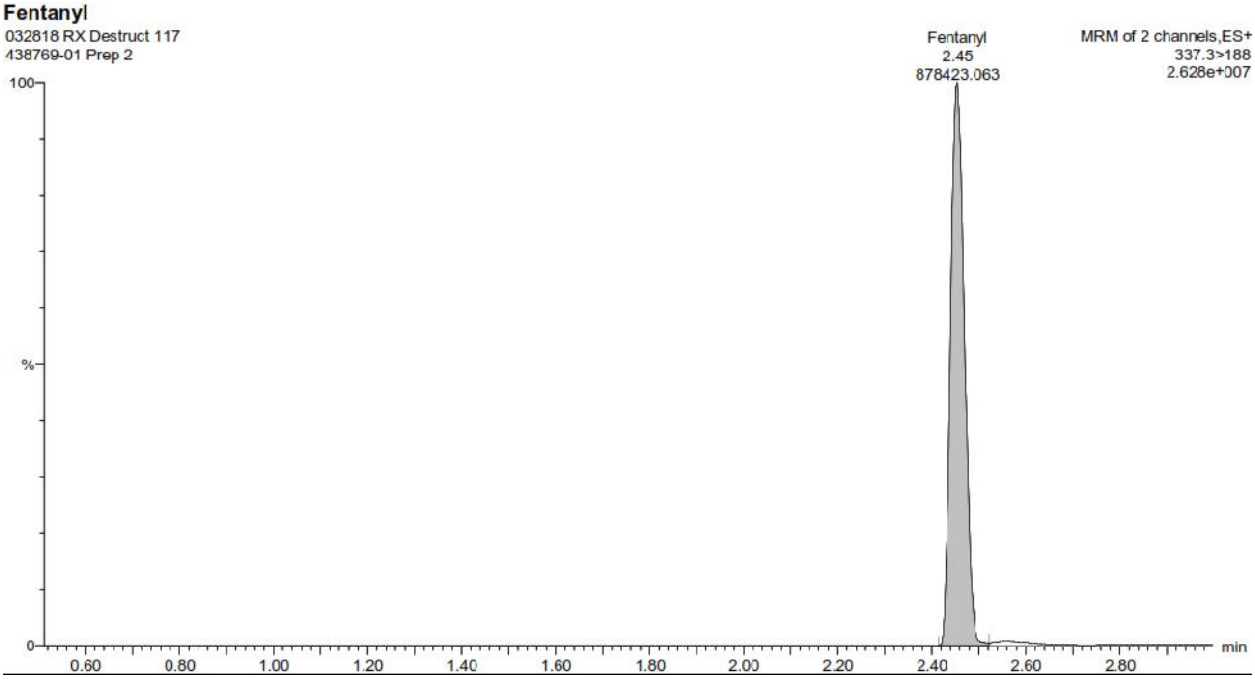


Figure 2: 1 mcg/mL Fentanyl Post-destruct Chromatogram (Filtered)

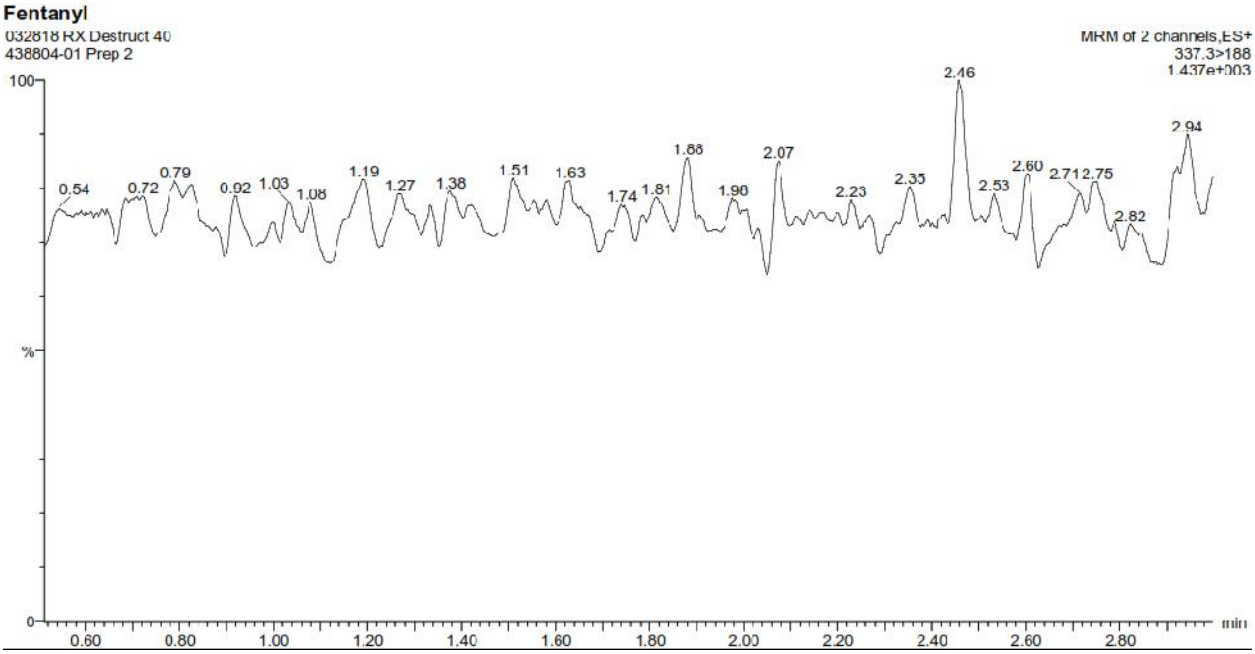


Table 1. Results for Pre-Destruction and Post-Destruction Fentanyl Samples

Fentanyl Sample Results: Pre-Destruction and Post-Destruction			
Sample Description	**Labeled Fentanyl Concentration (Pre-Destruction)***	**Average Peak Area (counts; n=3)**	**% Of Labeled Pre-Destruct Fentanyl Concentration*** **
1 mcg/mL Pre-Destruction Prep. #1	0.476 mcg/mL	902582	**Not Applicable**
1 mcg/mL Pre-Destruction Prep. #2		900785	
1 mcg/mL Pre-Destruction Prep. #3		917772	
1.5-2 mcg/mL Pre-Destruction Prep. #1	~0.832 mcg/mL**	927196	
1.5-2 mcg/mL Pre-Destruction Prep. #2		896689	
1.5-2 mcg/mL Pre-Destruction Prep. #3		927834	
1 mcg/mL Post-Destruction (Filtered) Prep. #1	0.476 mcg/mL	Below LOD	Less Than 0.0034%
1 mcg/mL Post-Destruction (Filtered) Prep. #2		Below LOQ	Less Than 0.0420%
1 mcg/mL Post-Destruction (Filtered) Prep. #3		Below LOQ	Less Than 0.0420%
1.5-2 mcg/mL Post-Destruction (Filtered) Prep. #1	~0.832 mcg/mL**	Below LOD	Less Than 0.0019%
1.5-2 mcg/mL Post-Destruction (Filtered) Prep. #2		Below LOD	Less Than 0.0019%
1.5-2 mcg/mL Post-Destruction (Filtered) Prep. #3		Below LOD	Less Than 0.0019%
1.5-2 mcg/mL Post-Destruction (Un-Filtered) Prep. #1	~0.832 mcg/mL**	Below LOQ	Less Than 0.0240%
1.5-2 mcg/mL Post-Destruction (Un-Filtered) Prep. #2		Below LOQ	Less Than 0.0240%
1.5-2 mcg/mL Post-Destruction (Un-Filtered) Prep. #3		Below LOQ	Less Than 0.0240%

* Label Concentration was calculated using the sample dilution during RxDestruct Process. See "Pre-Destruct and Post-Destruct Sample Preparations" section.

**For calculations, the average (1.75 mcg/mL) of the range of initial concentration of sample described as 1.5 - 2 mcg/mL Fentanyl was used.

***This value was calculated using the reported LOQ or LOD, and the Pre-Destruction Concentration of Drug. E.g. for 1 mcg/mL Post-Destruction (Filtered) Prep. #1 = (LOD Concentration / Pre-Destruction Concentration) x 100 = (0.000016 mcg/mL / 0.476 mcg/mL) x 100 = 0.0034% of Pre-Destruct Concentration

Figure 3: 8 mcg/mL Hydromorphone Pre-destruct Chromatogram

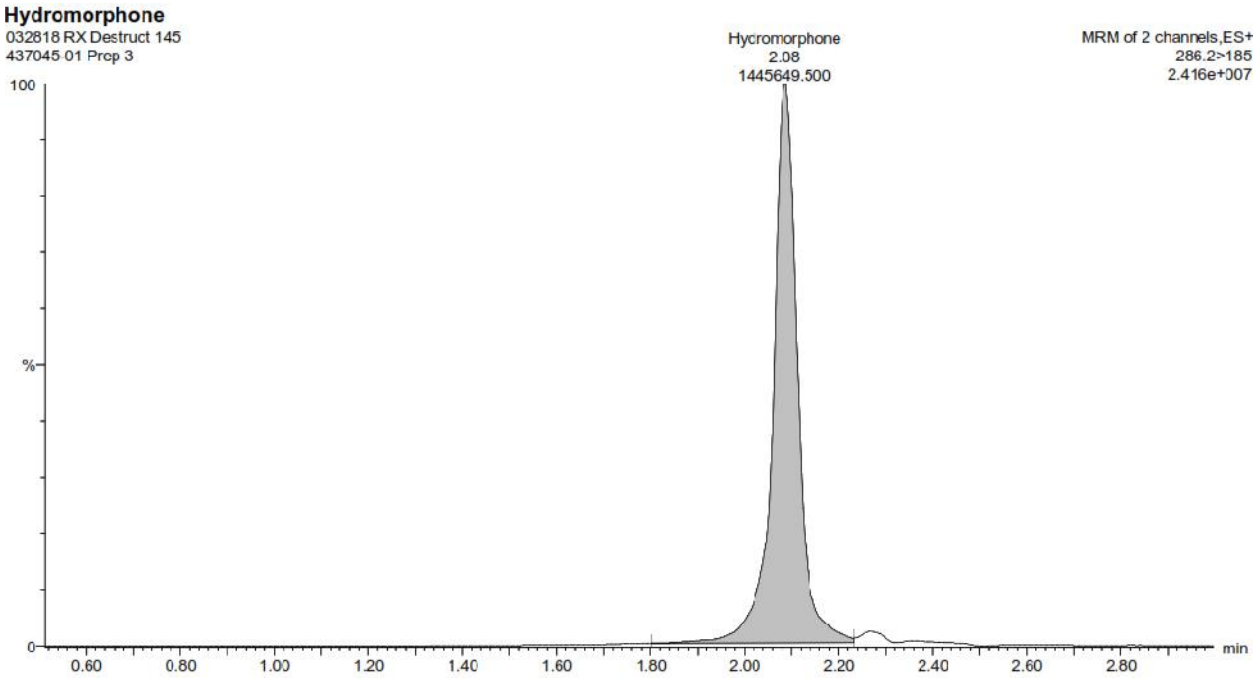


Figure 4: 8 mcg/mL Hydromorphone Post-destruct Chromatogram (Filtered)

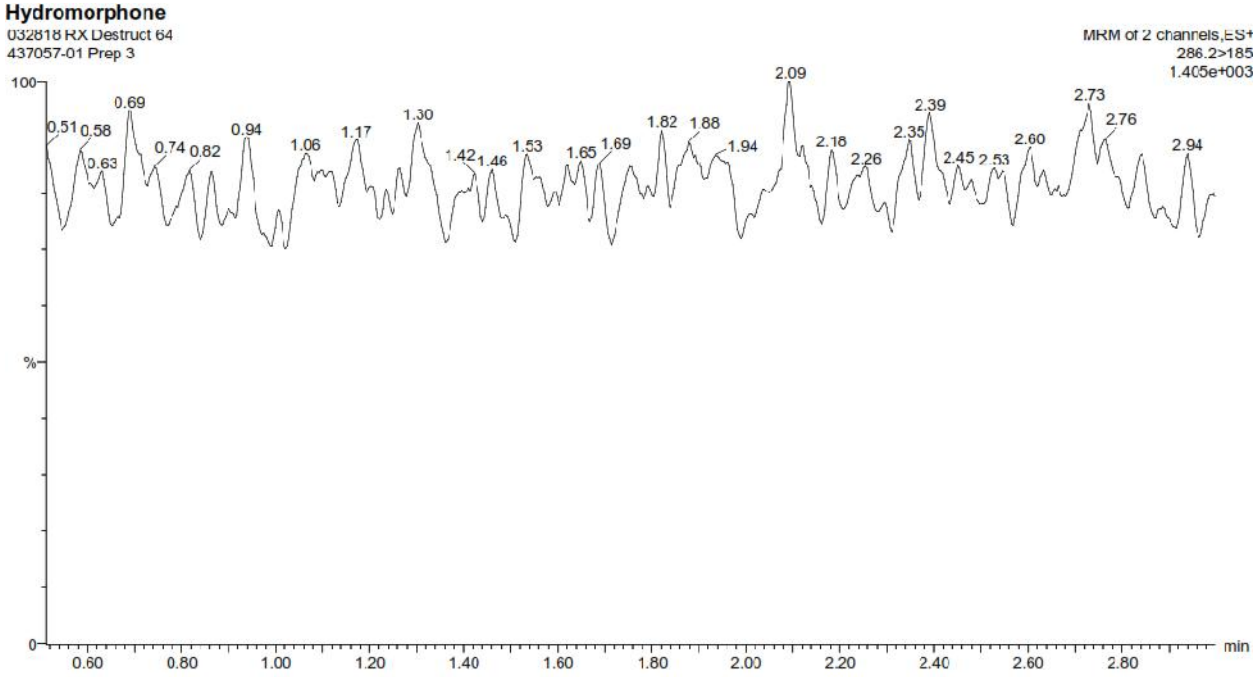


Table 2: Results for Pre-Destruction and Post-Destruction Hydromorphone HCl Samples

Hydromorphone Sample Results: Pre-Destruction and Post-Destruction			
Sample Description	**Labeled Hydromorphone HCl Concentration (Pre-Destruction)***	**Average Peak Area (counts; n=3)**	**% Of Labeled Pre-Destruct Hydromorphone HCl Concentration****
8 mcg/mL Pre-Destruction Prep. #1	3.81 mcg/mL	1418270	**Not Applicable**
8 mcg/mL Pre-Destruction Prep. #2		1422830	
8 mcg/mL Pre-Destruction Prep. #3		1420890	
12 mcg/mL Pre-Destruction Prep. #1	5.71 mcg/mL	435782	
12 mcg/mL Pre-Destruction Prep. #2		429291	
12 mcg/mL Pre-Destruction Prep. #3		424074	
8 mcg/mL Post-Destruction (Filtered) Prep. #1	3.81 mcg/mL	Below LOD	Less Than 0.0160%
8 mcg/mL Post-Destruction (Filtered) Prep. #2		Below LOD	Less Than 0.0160%
8 mcg/mL Post-Destruction (Filtered) Prep. #3		Below LOD	Less Than 0.0160%
12 mcg/mL Post-Destruction (Filtered) Prep. #1	5.71 mcg/mL	Below LOD	Less Than 0.0107%
12 mcg/mL Post-Destruction (Filtered) Prep. #2		Below LOD	Less Than 0.0107%
12 mcg/mL Post-Destruction (Filtered) Prep. #3		Below LOD	Less Than 0.0107%
8 mcg/mL Post-Destruction (Un-Filtered) Prep. #1	3.81 mcg/mL	Below LOD	Less Than 0.0160%
8 mcg/mL Post-Destruction (Un-Filtered) Prep. #2		Below LOD	Less Than 0.0160%
8 mcg/mL Post-Destruction (Un-Filtered) Prep. #3		Below LOD	Less Than 0.0160%
12 mcg/mL Post-Destruction (Un-Filtered) Prep. #1	5.71 mcg/mL	Below LOD	Less Than 0.0107%
12 mcg/mL Post-Destruction (Un-Filtered) Prep. #2		Below LOD	Less Than 0.0107%
12 mcg/mL Post-Destruction (Un-Filtered) Prep. #3		Below LOD	Less Than 0.0107%

* Label Concentration was calculated using the sample dilution during RxDestruct Process . See "Pre-Destruct and Post-Destruct Sample Preparations" section.

**This value was calculated using the reported LOQ or LOD, and the Pre-Destruction Concentration of Drug. E.g. for 8 mcg/mL Post-Destruction (Filtered) Prep. #1 = (LOD Concentration / Pre-Destruction Concentration) x 100 = (0.00061 mcg/mL / 3.81 mcg/mL) x 100 = 0.0160% of Pre-Destruct Concentration

The parameters measured in the validation of this method were:

- Limit of Quantitation and Limit of Detection (LOQ and LOD)
- System Suitability
- Specificity

Included in each section is the method used, criteria for acceptance, and results. All supporting data and chromatograms are included in the attachments.

Project #/ARL #

The method validation for Fentanyl and Hydromorphone HCl in this report were assigned ARL project # 0717-17 and ARL numbers 437045, 437053, 437057, 437064, 437067, 437071, 438769, 438804, 438814, 438821, and 438823.

VALIDATION TEST	NOTEBOOK PAGES IN NOTEBOOK
LOQ and LOD	DB1917 pgs. 130-139
System Suitability	DB1917 pgs. 130-139
Specificity	DB1917 pgs. 130-139

METHOD

Summary

An HPLC–MS method was employed for the separation and detection of Fentanyl and Hydromorphone HCl in Normal Saline, after exposure to Fenton's Reagent.

Equipment/Material

Column: C#4123, Phenomenex Kinetex Biphenyl, 2.6 µm, 30 mm x 2.1 mm.
HPLC: Agilent 1100 Series, HPLC#24, Calibration due 07/25/18
Mass Spectrometer: ARL MS-3, Calibration due 01/24/19
Software: Waters Masslynx V 4.1
Balance: ARL # 1232, Mettler Toledo, Calibration due 08/30/18
Micropipettes:

- ARL#103, 10-100 µL, Calibration Due 08/30/18
- ARL#123, 100-1000 µL, Calibration Due 08/30/18

Freezer: ARL # 471, Calibration due 08/30/18
Freezer: ARL # 1270, Calibration due 08/30/18

Reagents

Acetonitrile (ACN): R# 6793, HPLC Grade, Fisher Lot#173599, Expires 03/12/23
Formic Acid (FA): R# 6485, Fisher Lot#17305, Expires 08/31/22

Standards and Samples

Fentanyl: S#15920, U.S. Pharmacopeia Lot# R05790, Current Lot
Hydromorphone HCl: S# 15630, Letco Lot# 1609200004, Expires 05/31/19.
437045: Clear River Enviro, Inc.Hydromorphone 8 mcg/mL in NS - Before Destruction (Bag 1)
437053:Clear River Enviro, Inc. Hydromorphone 8 mcg/mL in NS-After Destruction NOT FILTERED (Bag 1)
*437057:*Clear River Enviro, Inc. Hydromorphone 8 mcg/mL in NS-After Destruction FILTERED (Bag 1)
437064: Clear River Enviro, Inc. Hydromorphone 12 mcg/mL in NS - Before Destruction (Bag 2)
437067: Clear River Enviro, Inc. Hydromorphone 12 mcg/mL in NS - After Destruction NOT FILTERED (Bag 2)
437071:Clear River Enviro,Inc. Hydromorphone 12 mcg/mL in NS-After Destruction FILTERED(Bag 2)
438769: Clear River Enviro, Inc. Fentanyl 1 mcg/mL - Before Destruction (Bag 1)
438804: Clear River Enviro, Inc. Fentanyl 1 mcg/mL - After Destruction FILTERED (Bag 1)
438814: Clear River Enviro, Inc. Fentanyl 1.5-2.0 mcg/mL - Before Destruction (Bag 2)
438821: Clear River Enviro, Inc. Fentanyl 1.5-2.0 mcg/mL - After Destruction NOT FILTERED (Bag 2)
438823: Clear River Enviro, Inc. Fentanyl 1.5-2.0 mcg/mL - After Destruction FILTERED (Bag 2)

Chromatographic Conditions

Mobile phase A: Combine 1000 mL of Nanopure Water and 5 mL of Formic Acid. Stir to mix, filter using 0.22 µm filter element, and de-gas.

Mobile phase B: Combine 1000 mL of Acetonitrile and 5 mL of Formic Acid. Stir to mix, filter using 0.22 µm filter element, and de-gas.

Diluent: Nanopure Water.

Column Temp: 45˚C
Autosampler Temperature: 4˚C
Flow Rate: 0.650 mL/min
Injection Volume: 20 ⇑L
Detection: Mass Spectrometer
Run Time: 5 minutes
Elution: Gradient

Time (min)	% Mobile Phase A	% Mobile Phase B
0.0	100	0
0.4	100	0
1.4	10	90
2.5	10	90
2.6	100	0
5.0	100	0

Mass Spectrometer Parameters:

Operating Parameter	Setting	Operating Parameter	Setting
Polarity	Positive	LM 1 Resolution	14.0
Fentanyl Transition:	286.2 → 185.0	HM 1 Resolution	14.0
Hydromorphone Transition:	337.3 → 188.0	Ion Energy 1	0.5
Capillary (kV)	4.00	Entrance	20
Cone (V)	50	Collision	25
Extractor (V)	5	Exit	5
RF Lens (V)	0.0	LM 2 Resolution	12.0
Source Temp (^{o}C)	120	HM 2 Resolution	12.0
Desolvation Temp. (^{o}C)	400	Ion Energy 2	1.0
Cone Gas Flow (L/hr)	50	Multiplier (V)	700
Desolvation Gas Flow (L/hr)	700	Collision Gas Flow (mL/min)	0.30

Instrumental Data Acquisition Methods

All standards and samples were analyzed with the same chromatographic conditions and mass spectrometer parameters.

Future analysis of Fentanyl or Hydromorphone HCl solutions in normal saline, after exposure to Fenton's reagent through the RxDestruct Device, will follow the method described herein.

Pre-Destruct and Post-Destruct Sample Preparations:

The RxDestruct process causes dilution of starting drug product. The stated pre-destruct concentration of samples is the concentration in 500 mL IV bags. This initial volume is diluted to a final volume of 1051 mL during the process. For example, the 8 mcg/mL initial concentration of Hydromorphone, after dilution resulting from the RxDestruct process, final concentration would be calculated by: 8 mcg/mL x 500 mL / 1051 mL, or 3.81 mcg/mL.

All Samples were diluted 1:10 in water prior to injection to decrease the possibility of remaining reagents from Fenton's reaction causing damage to the instrument or column. The reported results for the LOD and the LOQ factor in this dilution. If it can be demonstrated that neat injection of sample will not damage equipment, and will not cause suppression of the signal due to matrix effects, then a further ten-fold increase in sensitivity is possible.

Additionally, after 1:10 dilution of post-destruction samples described as "un-filtered" the resulting solution was passed through a 0.45 µm syringe filter. A filter check was performed to demonstrate recovery of both Fentanyl and Hydromorphone HCL by passing LOQ solutions of both drugs through a 0.45 µm syringe filter and comparing to unfiltered LOQ results.

Of note, the peak areas for the pre-destruct 8 mcg/mL Hydromorphone sample are greater than those for the pre-destruct 12 mcg/mL Hydromorphone sample. The source of this discrepancy is unknown.

SUMMARY OF VALIDATION DATA AND RESULTS FOR FENTANYL

TEST PERFORMED	CRITERIA	RESULTS
LOQ and LOD	LOQ: Prepare a solution of drug, with excipients, at a concentration where each of 6 replicate injections has a Signal to Noise (S/N) ratio of at least 10 and the %RSD of the six injections is ≤ 20%. LOD: Estimate LOD at a concentration where S/N ratio is approximately 3, based on the LOQ S/N result.	LOQ = 0.0002 mcg/mL S/N of each injection Not Less Than 10 %RSD of LOQ injections = 11% Estimated LOD = 0.000016 mcg/mL
System Suitability	% RSD ≤ 20% for six injections of calibration standard without excipients. S/N Ratio is not less than 10 for any injection	% RSD = 7% S/N of each injection Not Less Than 10
Specificity	No Interference between API and excipient peaks.	No Interference

SUMMARY OF VALIDATION DATA AND RESULTS FOR HYDROMORPHONE HCL

TEST PERFORMED	CRITERIA	RESULTS
LOQ and LOD	LOQ: Prepare a solution of drug, with excipients, at a concentration where each of 6 replicate injections has a Signal to Noise (S/N) ratio of at least 10 and the %RSD of the six injections is ≤ 20% LOD: Estimate LOD at a concentration where S/N ratio is approximately 3, based on the LOQ S/N result.	LOQ = 0.01 mcg/mL S/N of each injection Not Less Than 10 %RSD of LOQ injections = 20% Estimated LOD = 0.00061 mcg/mL
System Suitability	% RSD ≤ 20% for six injections of calibration standard without excipients. S/N Ratio is not less than 10 for any injection.	% RSD = 8% S/N of each injection Not Less Than 10
Specificity	No Interference between API and excipient peaks.	No Interference

LIMIT OF QUANTITATION AND LIMIT OF DETECTION

Prepare separate solutions of Fentanyl and Hydromorphone HCl, with excipients. Further dilute these solutions 1:10, and inject onto the system six times at the concentration where the following criteria are met.

Criteria for Acceptance

Limit of Quantitation (LOQ):

- Signal to Noise ratio (S/N) of each of 6 replicate injections of LOQ preparation is not less than 10
- %RSD of the 6 replicate injections of LOQ preparation is less than 20%

Limit of Detection (LOD):

- Estimate LOD at a concentration where S/N ratio is approximately 3, based on the LOQ S/N result.

Results

Figure 5: Example Chromatogram of Fentanyl LOQ Preparation (0.0002 mcg/mL)

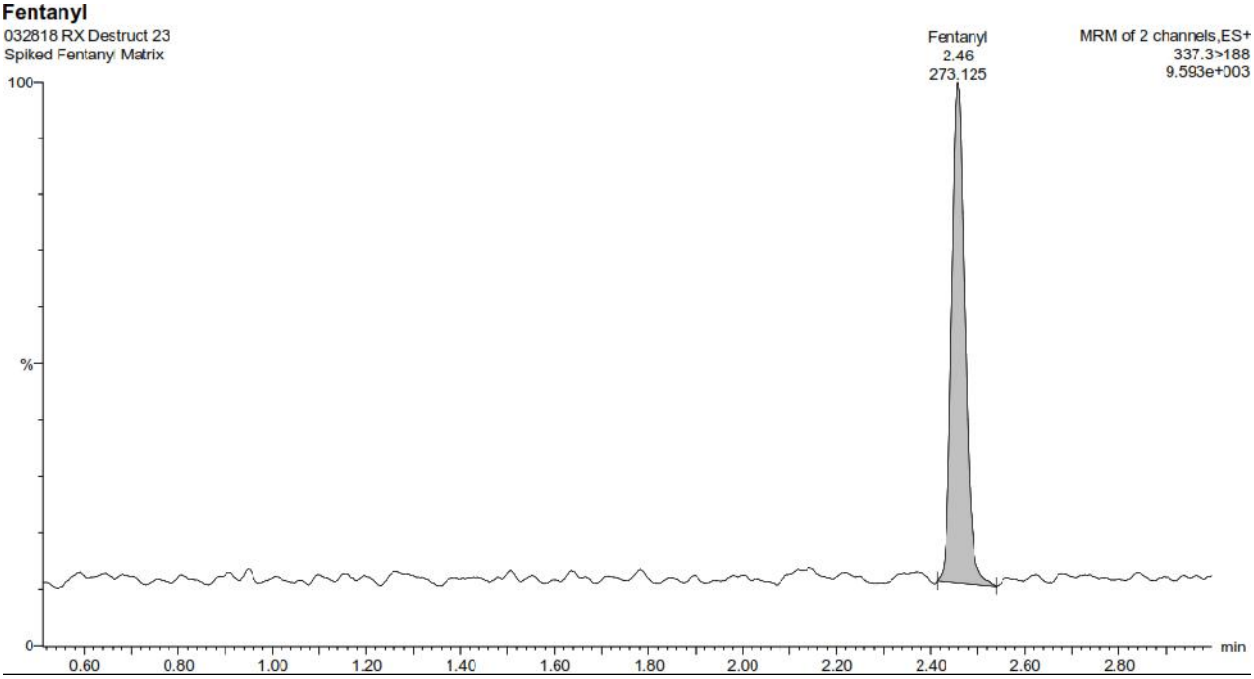


Figure 6: Example Chromatogram of Hydromorphone HCl LOQ Preparation (0.01 mcg/mL)

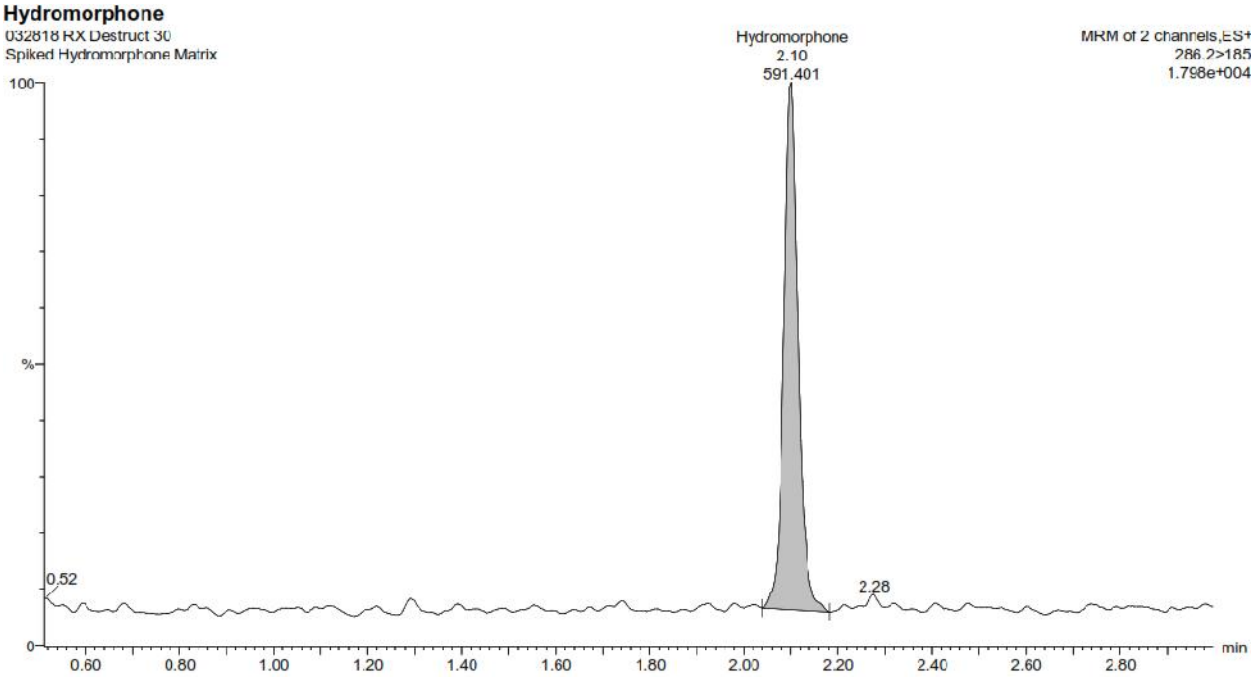


Table 3: LOQ Results for Fentanyl

Fentanyl Limit of Quantitation							
Sample Description	**Fentanyl Concentration**	**Analyte Peak Area (counts)**	**Analyte Retention Time (min)**	**Average Peak Area (counts)**	**Standard Deviation**	**% RSD**	**Signal to Noise Ratio**
Spiked Fentanyl Injection #1	0.0002 mcg/mL	257.814	2.46	246.389	26.631	**11%**	**37.95**
Spiked Fentanyl Injection #2		212.620	2.46				**40.46**
Spiked Fentanyl Injection #3		273.125	2.46				**35.53**
Spiked Fentanyl Injection #4		252.971	2.46				**35.4**
Spiked Fentanyl Injection #5		213.913	2.46				**29.26**
Spiked Fentanyl Injection #6		267.891	2.46				**40.84**

Table 4: LOD Results for Fentanyl

Fentanyl Limit of Detection				
Sample Description	**Fentanyl Concentration**	**Signal to Noise Ratio**	**Average Signal to Noise Ratio**	**Estimated LOD (S/N of ~3)**
Spiked Fentanyl Injection #1	0.0002 mcg/mL	**37.95**	**36.57**	0.000016 mcg/mL
Spiked Fentanyl Injection #2		**40.46**		
Spiked Fentanyl Injection #3		**35.53**		
Spiked Fentanyl Injection #4		**35.40**		
Spiked Fentanyl Injection #5		**29.26**		
Spiked Fentanyl Injection #6		**40.84**		

Table 5: LOQ Results for Hydromorphone HCl

Hydromorphone HCl Limit of Quantitation							
Sample Description	**Hydromorphone HCl Concentration**	**Analyte Peak Area (counts)**	**Analyte Retention Time (min)**	**Average Peak Area (counts)**	**Standard Deviation**	**% RSD**	**Signal to Noise Ratio**
Spiked Hydromorphone Injection #1	0.01 mcg/mL	538.986	2.10	565.240	30.682	**20%**	**47.92**
Spiked Hydromorphone Injection #2		598.300	2.10				**45.23**
Spiked Hydromorphone Injection #3		591.401	2.10				**56.73**
Spiked Hydromorphone Injection #4		529.320	2.10				**47.48**
Spiked Hydromorphone Injection #5		568.193	2.10				**67.59**
Spiked Hydromorphone Injection #6		316.173	2.13				**32.25**

Table 6: LOD Results for Hydromorphone HCl

Hydromorphone HCl Limit of Detection				
Sample Description	**Hydromorphone HCl Concentration**	**Signal to Noise Ratio**	**Average Signal to Noise Ratio**	**Estimated LOD (S/N of ~3)**
Spiked Hydromorphone Injection #1	0.01 mcg/mL	**47.92**	49.53	0.00061 mcg/mL
Spiked Hydromorphone Injection #2		**45.23**		
Spiked Hydromorphone Injection #3		**56.73**		
Spiked Hydromorphone Injection #4		**47.48**		
Spiked Hydromorphone Injection #5		**67.59**		
Spiked Hydromorphone Injection #6		**32.25**		

Conclusions

For Fentanyl, the criteria for limit of quantitation are met at a concentration of 0.0002 mcg/mL, and the estimated limit of detection is 0.000016 mcg/mL. For Hydromorphone HCl, the criteria for limit of quantitation are met at a concentration of 0.01 mcg/mL, and the estimated limit of detection is 0.00057 mcg/mL.

SYSTEM SUITABILITY

Prepare a solution containing both Fentanyl and Hydromorphone HCl, without excipients, at concentrations where the following criteria are met.

Criteria for Acceptance

- % RSD is ≤ 20% for six replicate injections of System Suitability Solution.
- Signal to Noise Ratio is not less than 10 for any injection

Results

Figure 7: Example System Suitability Chromatogram

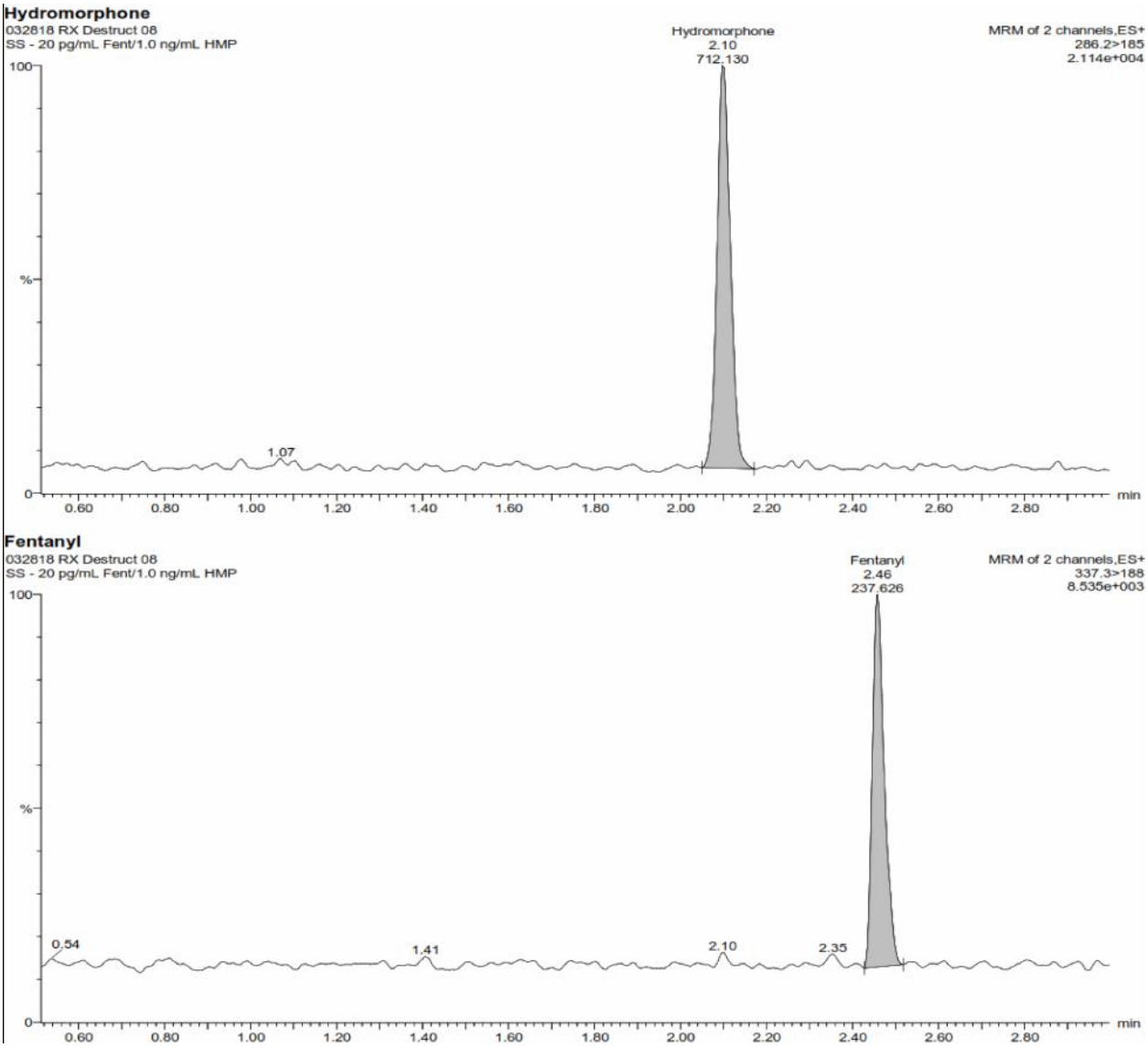


Table 7. System Suitability Results for Fentanyl

System Suitability - Fentanyl							
System Suitability Injection	**Fentanyl Concentration**	**Analyte Peak Area (counts)**	**Analyte Retention Time (min)**	**Average Peak Area (counts)**	**Standard Deviation**	**% RSD**	**Signal to Noise Ratio**
System Suitability Injection #1	0.00002 mcg/mL	237.626	2.46	212.588	14.560	**7%**	**44.23**
System Suitability Injection #2		199.205	2.46				**52.81**
System Suitability Injection #3		201.913	2.46				**43.82**
System Suitability Injection #4		221.514	2.46				**58.58**
System Suitability Injection #5		209.999	2.46				**78.30**
System Suitability Injection #6		205.268	2.46				**60.74**

Table 2. System Suitability Results for Hydromorphone HCl

System Suitability - Hydromorphone HCl							
System Suitability Injection	**Hydromorphone HCl Concentration**	**Analyte Peak Area (counts)**	**Analyte Retention Time (min)**	**Average Peak Area (counts)**	**Standard Deviation**	**% RSD**	**Signal to Noise Ratio**
System Suitability Injection #1	0.001 mcg/mL	712.130	2.10	693.876	57.175	**8%**	**44.23**
System Suitability Injection #2		745.905	2.10				**52.81**
System Suitability Injection #3		660.542	2.10				**43.82**
System Suitability Injection #4		682.542	2.10				**58.58**
System Suitability Injection #5		757.675	2.10				**78.30**
System Suitability Injection #6		604.464	2.11				**60.74**

Conclusion

The method passes for all system suitability requirements.

SPECIFICITY

Criteria for Acceptance

- No co-elution of analyte with expected excipients in chromatograms of Blank Matrices.

Results

There are no other peaks present in the chromatograms of blank matrix that co-elute with Fentanyl or Hydromorphone HCl.

Figure 8: Example Chromatogram of Blank Fentanyl Matrix

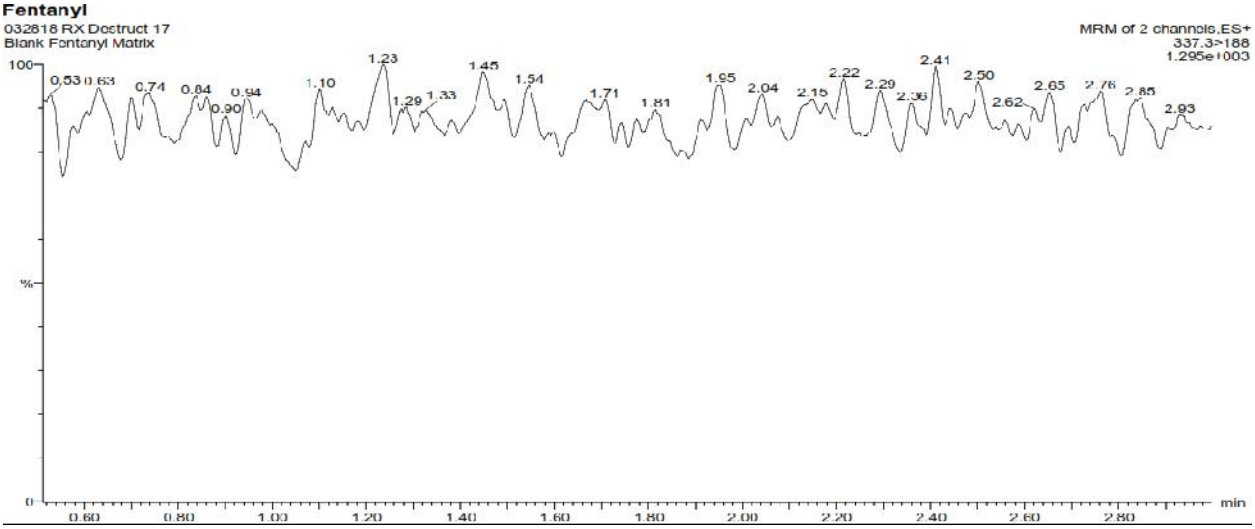
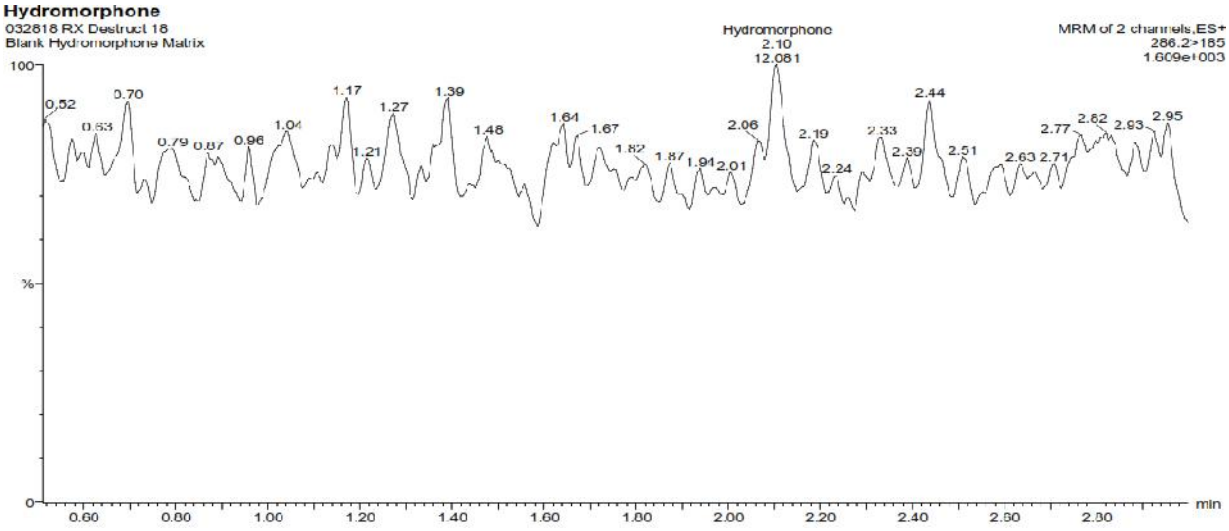


Figure 9: Example Chromatogram of Blank Hydromorphone HCl Matrix



Conclusions

This method demonstrates specificity for Fentanyl and Hydromorphone in solutions processed by the RxDestruct device.

OVERALL CONCLUSION

The method described herein meets all validation criteria for the detection of Fentanyl and Hydromorphone in normal saline solutions that have been processed by the RxDestruct device.