

Introduction

The analytical measurement of opioid panels commonly include a number of compounds such as heroin, methadone and buprenorphine. Research methods with sufficient flexibility are of value to respond to the need to measure new compounds resulting from clandestine production.

Desomorphine, also known by its street name Krokodil, is an emerging synthetic opioid, used as a heroin substitute and produced by back street illicit laboratories. Little or no literature exists presently describing an analytical approach to determining desomorphine in samples.

This poster describes the development of an LC/MS QQQ research method that aims to rapidly screen for to and measure desomorphine together with heroin, buprenorphine, methadone and their metabolites 6-acetylmorphine, norbuprenorphine and EDDP.

A three minute analytical research method has been developed with results obtained for five batches of spiked urine samples over the linear concentration range of 1-5000ng/ml. Precision data obtained, LLOQ, calibration linearity and accuracies will be reported for each analyte.

Sample Preparation

Spiked Urine Sample Preparation (Dilute & Shoot):

1. Internal Standard Diluent Preparation (per individual batch):

- Take 36ml of deionized water;
- Add 10uL of each internal standard (100ug/ml) or 1uL (1mg/ml) to the water;
- Vortex the ~ 25ng/ml ISTD solution.

2. Negative Urine Pre-treatment:

- Split 5ml of neat negative urine between 10x nanosep 3K centrifuge filters;
- Centrifuge @ 12000 for 20 min;
- Pool filtered urine into single tube or vessel;
- Draw 4ml of the filtered urine and add to the 36ml ISTD solution (1);
- This solution was used as calibrator diluent containing 25ng/ml of each ISTD.

Table 1 - Internal Standards Utilized (Cerilliant Corp.)

6-Monoacetylmorphine-D6	Heroin-D9
Buprenorphine-D4	Methadone-D9
Desomorphine-D3	Norbuprenorphine-D3
EDDP-D3	

HPLC Experimental Parameters

HPLC Method Conditions (Agilent 1290 system):

Column: Agilent Poroshell 120 EC C18, 2.1 x 50mm (2.7µm)
 Column temperature: 55°C
 Injection volume: 1 uL
 Autosampler temp: 4°C
 Needle wash: flushport (100% acetonitrile), 5 sec

Mobile phase: A = 5mM NH₄formate/0.01% formic acid in water
 B = 0.01% formic acid in methanol

Gradient flow rate: 0.5 mL/min

Experimental & Instrument Parameters

Table 2 - HPLC Gradient Profile.

Time (min)	% A	%B
0.00	90	10
1.10	75	25
1.60	5	95
2.00	5	95
2.01	90	10
3.00	90	10

Mass Spectrometer Conditions & Configuration:

Configuration:

Agilent 6460 QqQ Mass Spectrometer.

Ion Source Conditions:

Ion Mode: ESI/ Positive.
 Capillary Voltage: 2500 V
 Nozzle Voltage: 0 V
 Drying gas (nitrogen): 9 L/min
 Drying gas temperature: 250 °C
 Nebulizer gas (nitrogen): 35 psi
 Sheath Gas temperature: 380 °C
 Sheath Gas flow: 11 L/min

Table 3 - MRM acquisition Parameters:

Cpd Name	Prec Ion	Prod Ion	Dwell	Frag (V)	CE (V)	Cell Acc (V)
6-monoacetyl morphine	328.2	165.1	20	158	41	3
6-monoacetyl morphine	328.2	43.1	20	158	60	3
6-monoacetyl morphine-D6	334.2	211	20	168	25	3
Buprenorphine	468.3	83.7	35	210	53	3
Buprenorphine	468.3	55.1	35	210	65	3
Buprenorphine-D4	472.3	88.1	35	220	49	3
Buprenorphine-D4	472.3	59.1	35	220	61	3
Desomorphine	272.2	165.2	20	161	65	3
Desomorphine	272.2	152.2	20	161	65	3
Desomorphine-D3	275.2	165.2	20	156	65	3
Desomorphine-D3	275.2	152.2	20	156	65	3
EDDP	278.2	234.1	20	158	33	7
EDDP	278.2	219.1	20	158	45	7
EDDP-D3	281.2	234.1	20	168	29	7
Heroin	370.2	268.1	20	149	37	7
Heroin	370.2	165	20	149	61	7
Heroin-D9	379.2	61.2	20	168	29	7
Methadone	310.2	265.1	20	112	9	7
Methadone	310.2	105	20	112	29	7
Methadone-D9	319.3	268.1	20	89	13	7
Norbuprenorphine	414.3	83.1	35	178	57	3
Norbuprenorphine	414.3	55.2	35	178	65	3
Norbuprenorphine-D3	417.3	101.1	35	198	45	3
Norbuprenorphine-D3	417.3	83.1	35	198	57	3

Desomorphine Results

Figure 1 - Chromatography (1ng/ml) and Linear Dynamic Range (1-5000ng/ml.)

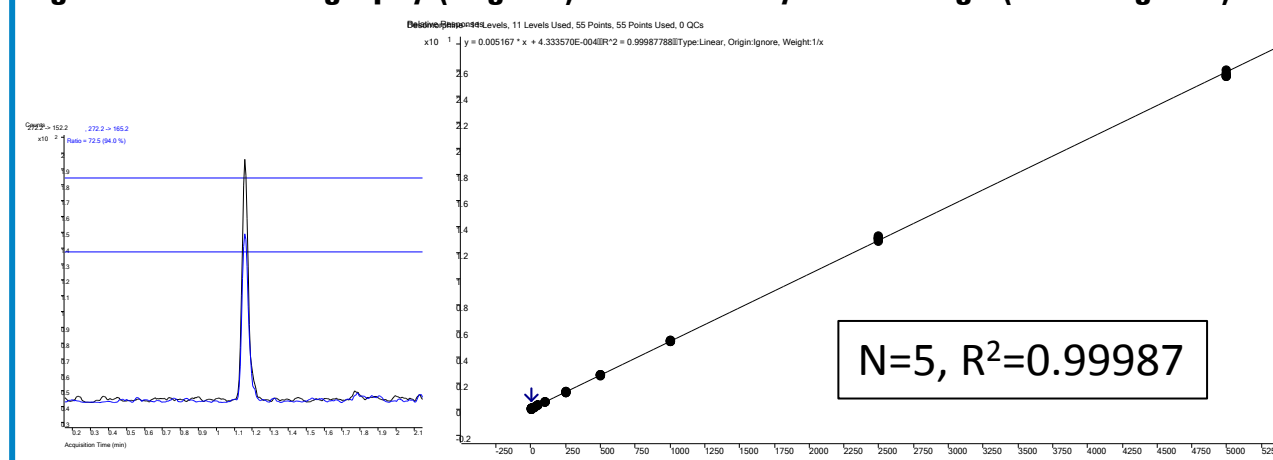
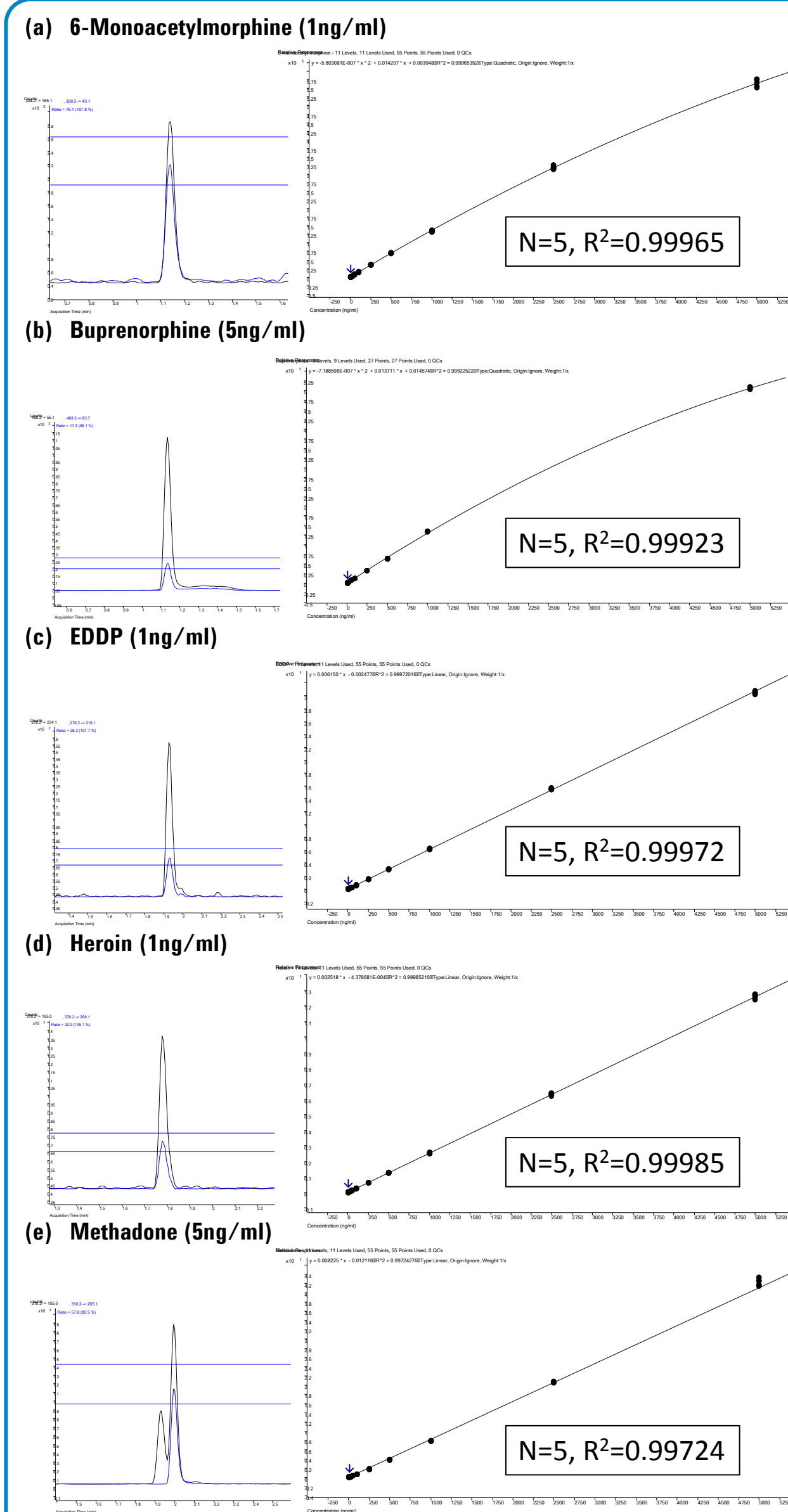


Table 4 - Precision Data for Desomorphine calibrator levels over N=5 Batches.

Concentration (ng/ml)	% RSD	Concentration (ng/ml)	%RSD
1	6.75	50	0.68
5	2.14	100	0.91
10	1.64	250	0.60
25	1.46	500	0.46

Results of Other Opiate Method Analytes

Quantitative Linearity of Opiate (1ng/ml→5000ng/ml)



Summary of Opiate Analytical Method Results:

- LOQ values for all opiates in this study were typically <1ng/ml (except Buprenorphine and was 5ng/ml.) These equate to a Urine equivalent of 10ng/ml since there was a 1/10 Urine dilution.
- Over five separately spiked batches of samples, %RSD values for desomorphine were <6.75% across the Linear range.
- This rapid three minute method illustrates the feasibility of distinguishing between illicit opiate usage including desomorphine.

Conclusions

The analytical method developed herein has demonstrated the feasibility of:

- Analyzing illicit opiate/opioids & metabolites directly and individually in a rapid three minute chromatographic method;
- Demonstrated the ability to detect desomorphine from a LOQ < 1ng/ml across a wide linear dynamic through at least 5000ng/ml;
- Illustrated that low limits of quantitation can be achieved for opiates and their metabolites with minimal sample preparation;
- Wide linear dynamic ranges of ~ 4 orders can be achieved for all analytes in this study.