

# Phase I Metabolism of Desomorphine

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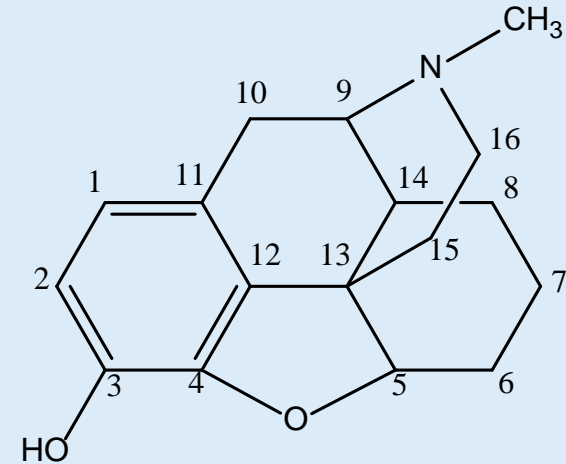
**Sam Houston  
State University**

# Disclosures & Acknowledgements

- This project was supported by Award No. 2015-R2-CX-0031 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.
- The authors have no commercial disclosures

# Desomorphine

- A semi-synthetic derivative of morphine
  - First synthesized in the early 1900s
- Narcotic analgesic
  - Mu receptor agonist
- Produced for a short time as a prescription pain killer
  - Permanently withdrawn from the market in 1952
  - Currently regarded worldwide as having no medical use
- Became a drug of abuse in the 2000s
  - Severe side effects include gangrene and necrotic ulcers



# Pharmacology

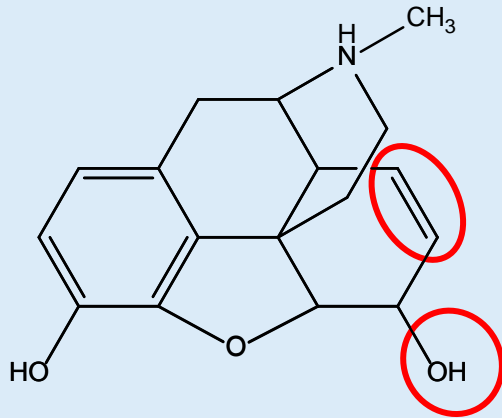
- Animals

- Desomorphine showed 10 times the depressant effects of morphine
- Those dosed with desomorphine experienced less vomiting than those dosed with morphine
- LD<sub>50</sub> of pure desomorphine was found to be 27 mg/kg, greater than heroin but much less than morphine and codeine

- Humans

- Duration of effect lasted no more than 3 hours regardless of dose and the typical abstinence syndrome of opioids observed

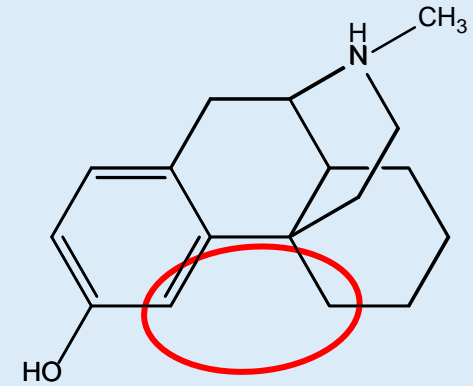
# Phenanthrene-Type Opioids



~~Morphine~~  
Morphine

CYP3A4

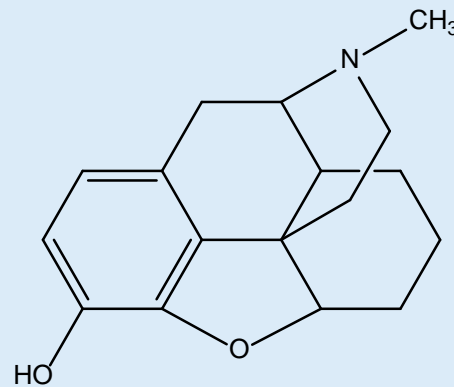
Projean et al 2003



~~Naloxone~~  
Naloxone

CYP3A4

Misra et al 1974



Desomorphine

# Metabolism

- **Richter et al 2016:** supersomes, human liver microsomes, human liver cytosol and rat studies
- Phase I metabolites identified in HLMs:
  - Nordesomorphine, desomorphine-*N*-oxide, 5 hydroxydesomorphine isomers
- Recombinant human P450s investigated:
  - rCYP1A2, rCYP2A4, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, rCYP2E1, rCYP3A4, rCYP3A5
- **Metabolic activity observed only in rCYP3A4**
  - Formed nordesomorphine, desomorphine-*N*-oxide, 3 hydroxydesomorphine isomers

# Reagents

## General Reaction

- Bactosomes – eight P450s
  - rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C18, rCYP2C19, rCYP2D6, and rCYP3A4
- Control bactosomes (no CYP gene)
- Blanks (enzyme added but no drug)
- 500  $\mu\text{L}$  reaction volume
  - 200  $\mu\text{M}$  desomorphine
  - 50 pmol/mL enzyme
  - 100  $\mu\text{M}$  pH 7.4 potassium phosphate buffer
  - 1.3 mM NADP+
  - 3.3 mM glucose-6-phosphate and magnesium citrate
  - 0.4 U/mL glucose-6-phosphate dehydrogenase

## Inhibitors

- Ketoconazole – rCYP3A4, rCYP2C9, rCYP2C18, rCYP2C19
  - 20  $\mu\text{M}$  in reaction
- Fluvoxamine – rCYP2D6, rCYP1A2
  - 20  $\mu\text{M}$  in reaction
- Ticlopidine – rCYP2C8
  - 10  $\mu\text{M}$  in reaction
- Montelukast – rCYP2B6
  - 10  $\mu\text{M}$  in reaction

# Incubation Setup

phosphate buffer (pH 7.4)  
desomorphine  
NADP+  
glucose-6-phosphate  
magnesium citrate  
glucose-6-phosphate dehydrogenase



**Uninhibited**  
N=3

phosphate buffer (pH 7.4)  
desomorphine  
NADP+  
glucose-6-phosphate  
magnesium citrate  
glucose-6-phosphate dehydrogenase  
inhibitor



**Inhibited**  
N=3

Pre-incubate 5 min at  
37°C then add enzyme

Time Stops: 0 and 4 hours  
Remove 25  $\mu$ L and add to 25  $\mu$ L of  
cold acetonitrile with 0.1% formic acid  
and 5  $\mu$ M desomorphine-D<sub>3</sub>

Centrifuge for 3 minutes (4°C and 10,000xg) and dilute  
supernatant 1:1 in 50:50 mobile phase (0.1% formic acid in  
water and 0.1% formic acid in acetonitrile)



# LC/Q-TOF-MS Conditions

## Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS

### LC Separation

- Poroshell 120 EC-C18 Column (2.1x100 mm, 2.7  $\mu\text{m}$ )
- Mobile Phase A: 0.1% FA in diH<sub>2</sub>O
- Mobile Phase B: 0.1% FA in ACN
- Flow Rate: 0.30 mL/min
- LC Gradient: 90% A (2 min), 63% A (6 min), 10% A (8 min)

### Q/TOF Parameters

- Gas Temperature: 150°C
- Gas Flow Rate: 13 L/min
- Sheath Gas Temperature: 200°C
- Sheath Gas Flow Rate: 12 L/min
- Nebulizer Pressure 45 psig

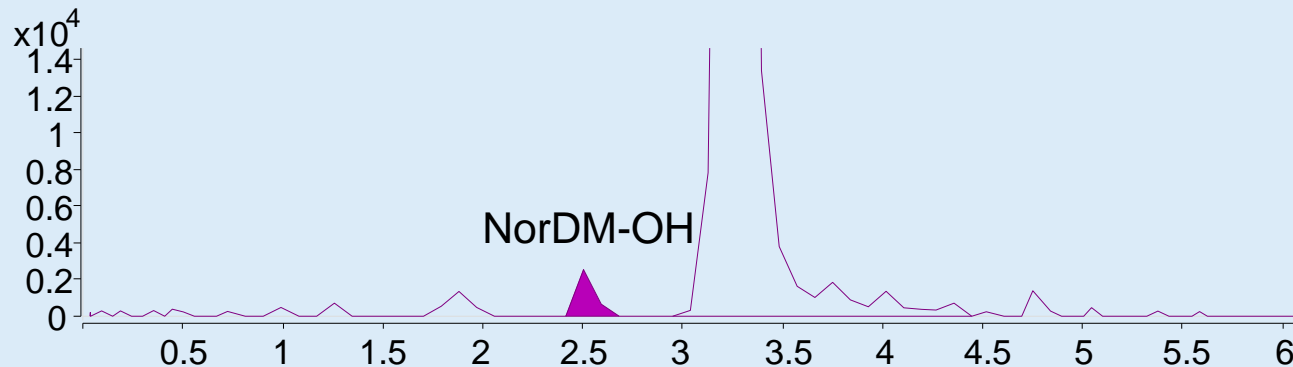
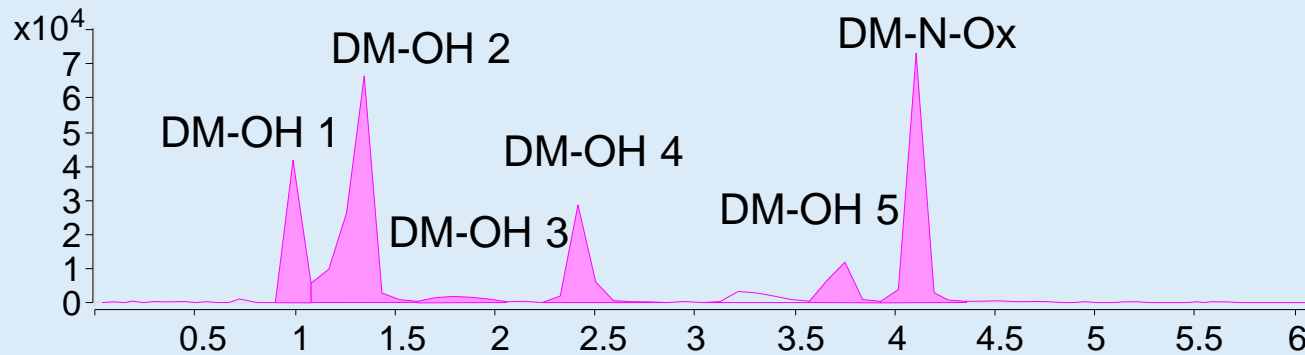
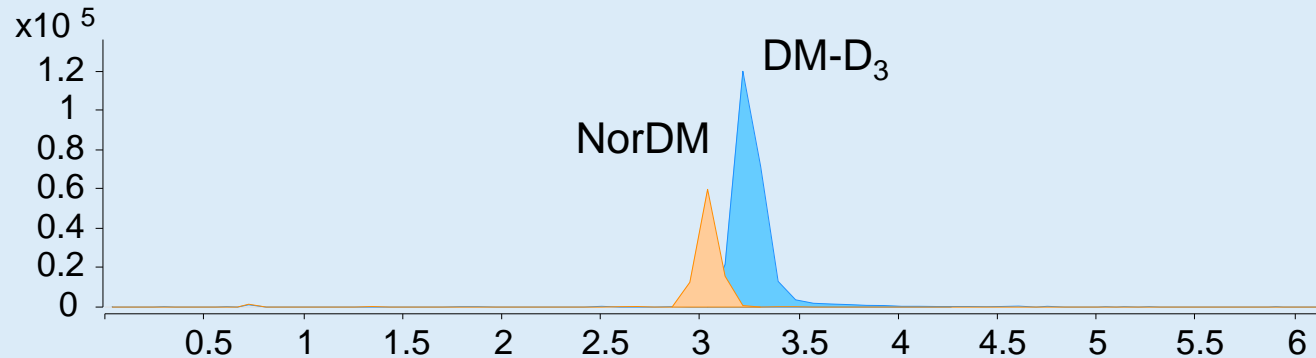
### Mass Spectrometry

- Capillary Voltage: 4000 V
- Fragmentor Voltage: 150 V
- Nozzle Voltage: 1000 V
- Collision Energy: 30 eV, 40 eV, 50 eV
- MS Scan Rate: 8 spectra/sec
- MS/MS Scan Rate: 3 spectra/sec
- MS Scan Range: 100-1000 m/z
- ESI Mode: Positive

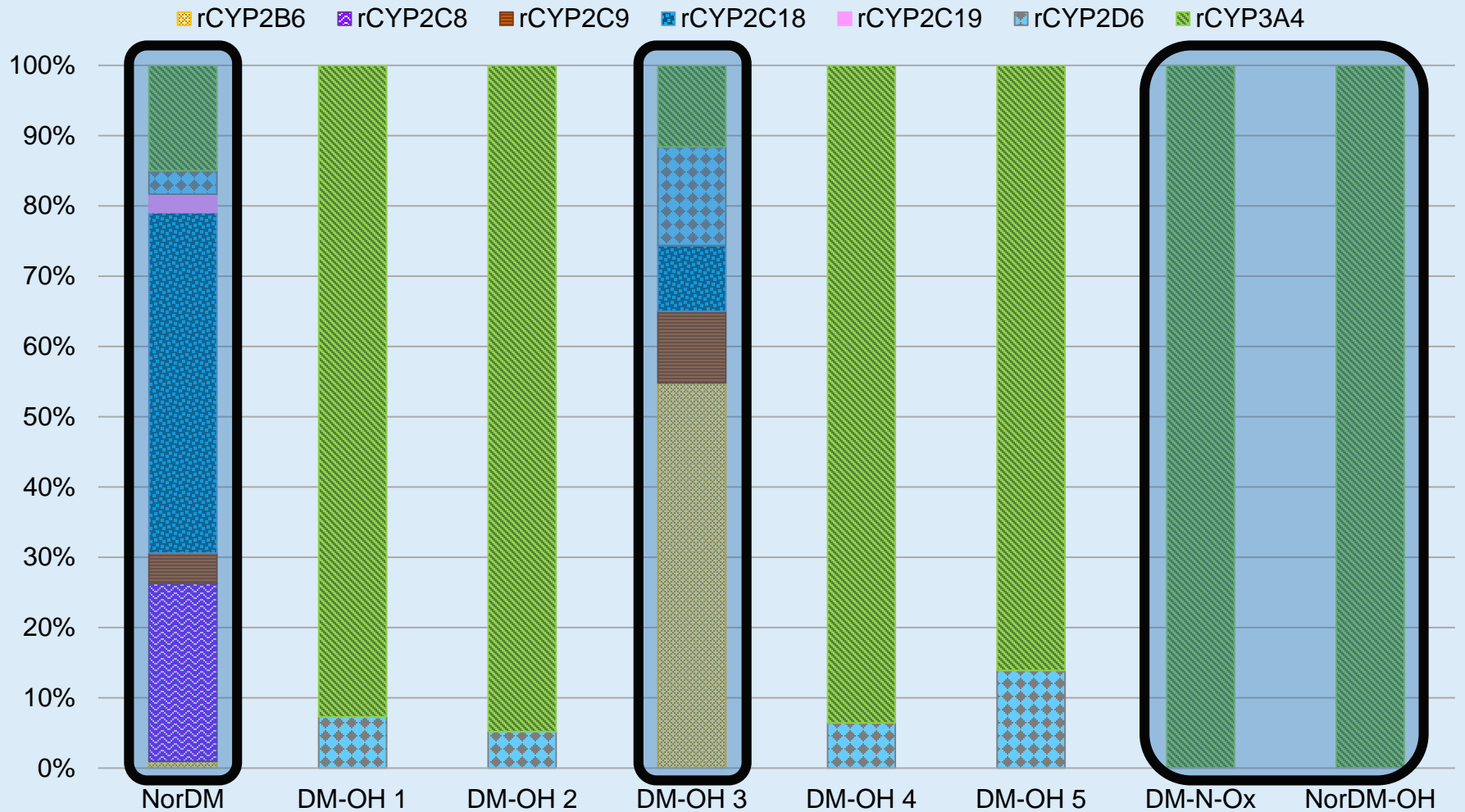
### Acquisition

- Full Scan Auto
- Run Time: 8 minutes

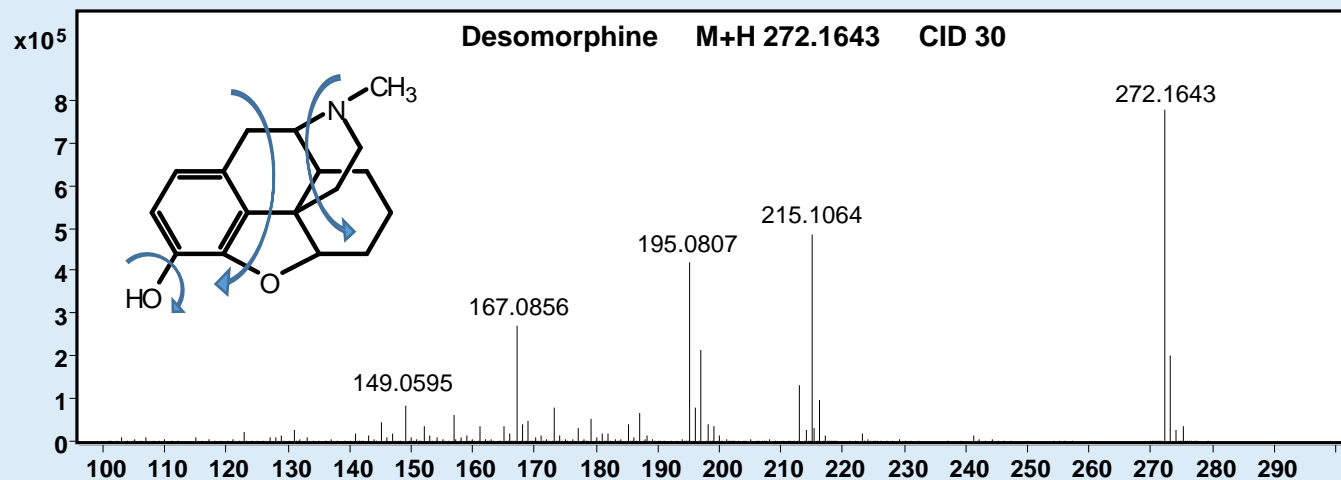
# Extracted Ion Chromatograms



# CYP Activity

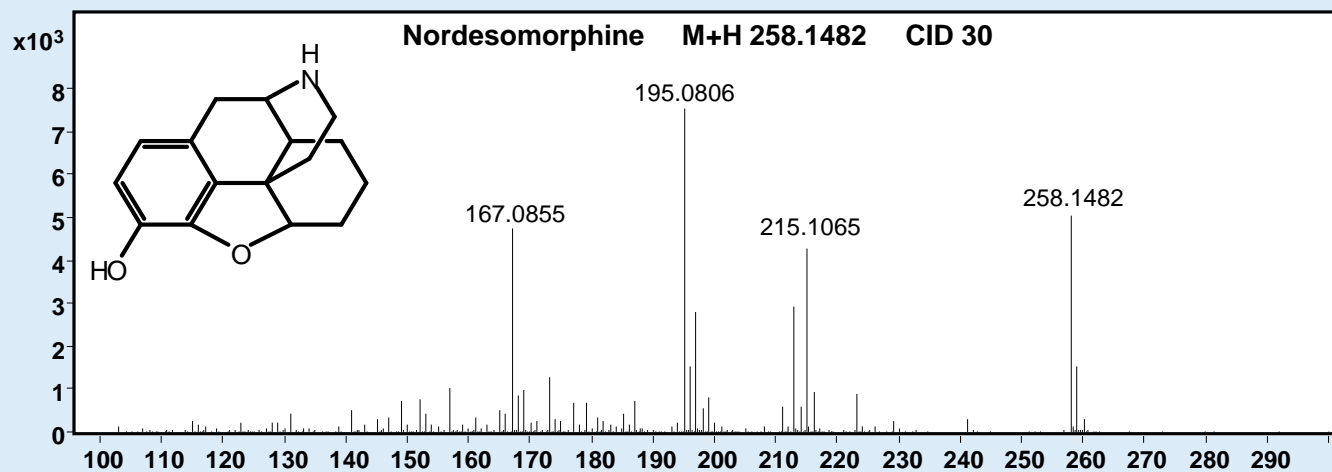


# Desomorphine MS<sup>2</sup>



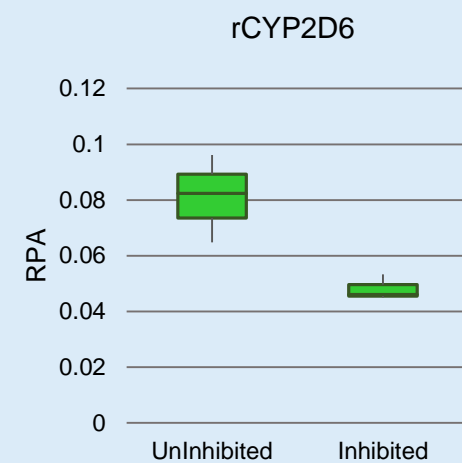
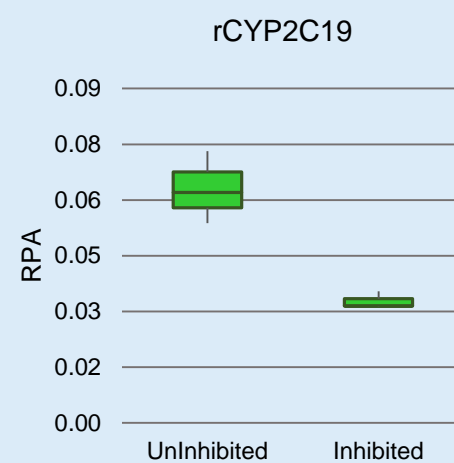
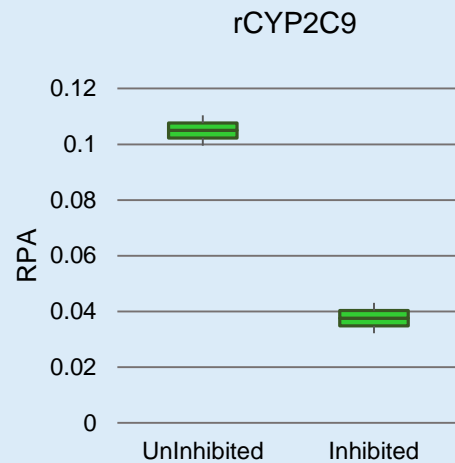
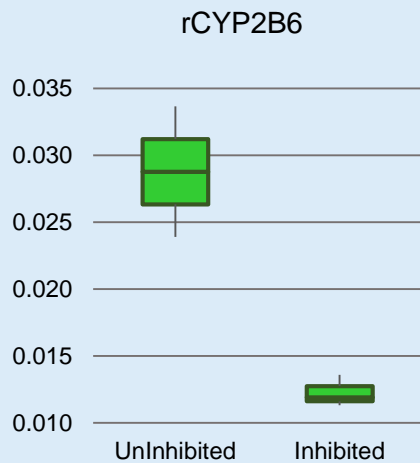
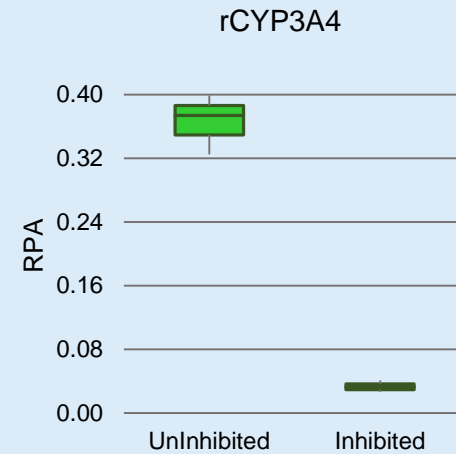
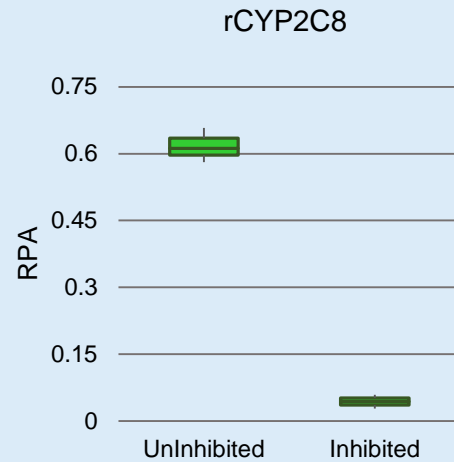
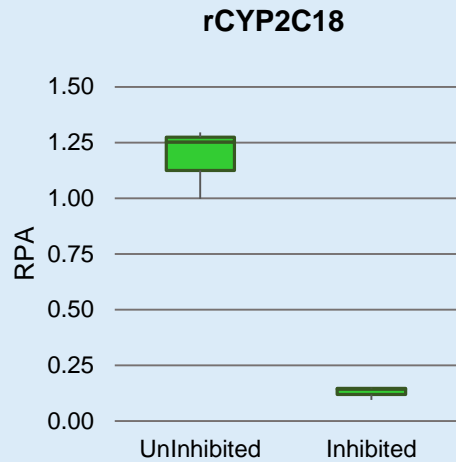
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
272	C <sub>17</sub> H <sub>22</sub> NO <sub>2</sub> <sup>+</sup>	272.1645	272.1643	0.91
215	C <sub>14</sub> H <sub>15</sub> O <sub>2</sub> <sup>+</sup>	215.1067	215.1064	1.00
195	C <sub>14</sub> H <sub>11</sub> O <sup>+</sup>	195.0804	195.0807	1.08
167	C <sub>13</sub> H <sub>11</sub> <sup>+</sup>	167.0855	167.0856	0.53
149	C <sub>9</sub> H <sub>9</sub> O <sub>2</sub> <sup>+</sup>	149.0597	149.0595	1.52

# Nordesomorphine MS<sup>2</sup>

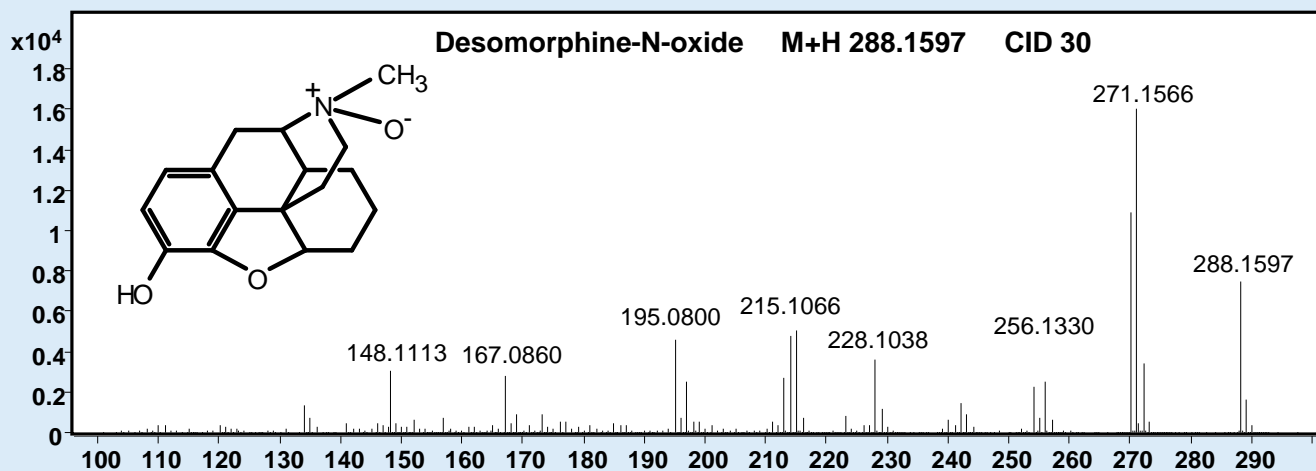


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
258	C <sub>16</sub> H <sub>20</sub> NO <sub>2</sub> <sup>+</sup>	258.1489	258.1482	2.54
215	C <sub>14</sub> H <sub>15</sub> O <sub>2</sub> <sup>+</sup>	215.1067	215.1065	0.74
195	C <sub>14</sub> H <sub>11</sub> O <sup>+</sup>	195.0804	195.0806	1.04
167	C <sub>13</sub> H <sub>11</sub> <sup>+</sup>	167.0855	167.0855	0.19

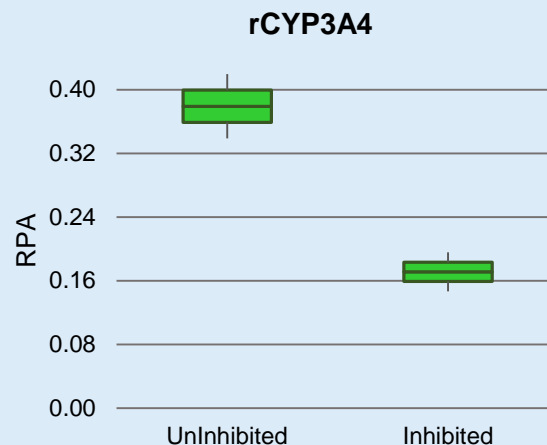
# Nordesomorphine



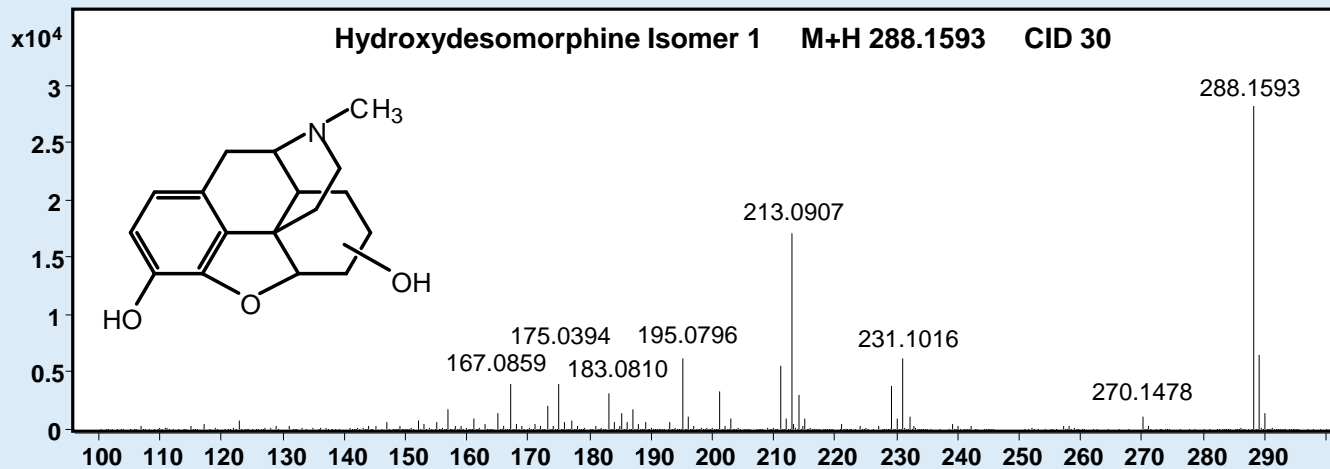
# Desomorphine-N-oxide MS<sup>2</sup>



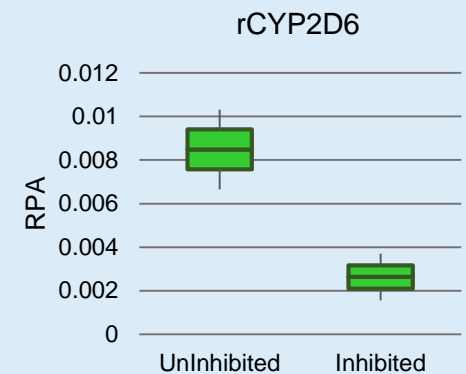
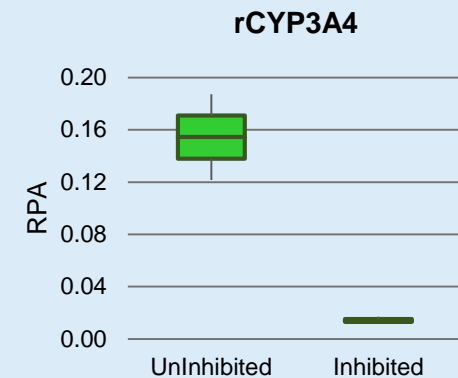
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	C <sub>17</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>	288.1594	288.1597	1.11
271	C <sub>17</sub> H <sub>21</sub> NO <sub>2</sub> <sup>+</sup>	271.1567	271.1566	0.27
256	C <sub>16</sub> H <sub>18</sub> NO <sub>2</sub> <sup>+</sup>	256.1332	256.1330	0.77
228	C <sub>14</sub> H <sub>14</sub> NO <sub>2</sub> <sup>+</sup>	228.1019	228.1038	8.34
215	C <sub>14</sub> H <sub>15</sub> O <sub>2</sub> <sup>+</sup>	215.1067	215.1066	0.42
195	C <sub>14</sub> H <sub>11</sub> O <sup>+</sup>	195.0804	195.0800	2.40
167	C <sub>13</sub> H <sub>11</sub> <sup>+</sup>	167.0856	167.0860	2.88
148	C <sub>10</sub> H <sub>14</sub> N <sup>+</sup>	148.1121	148.1113	5.46



# Hydroxydesomorphine Isomer 1 MS<sup>2</sup>

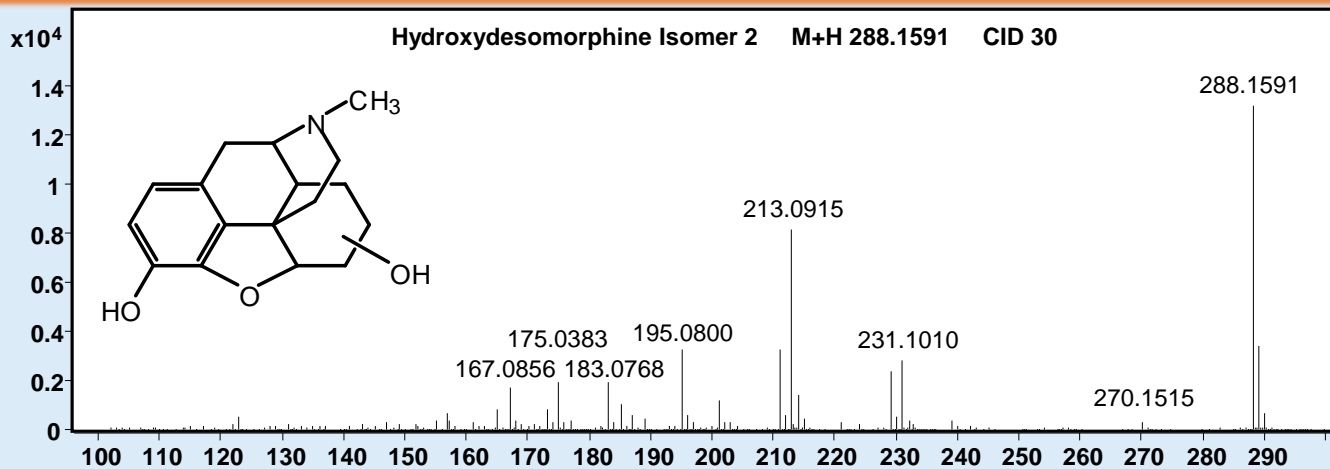


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	C <sub>17</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>	288.1594	288.1593	0.25
270	C <sub>17</sub> H <sub>20</sub> NO <sub>2</sub> <sup>+</sup>	270.1489	270.1478	3.78
231	C <sub>14</sub> H <sub>15</sub> O <sub>3</sub> <sup>+</sup>	231.1016	231.1016	0.06
213	C <sub>14</sub> H <sub>13</sub> O <sub>2</sub> <sup>+</sup>	213.0910	213.0907	1.40
195	C <sub>14</sub> H <sub>11</sub> O <sup>+</sup>	195.0804	195.0796	4.33
183	C <sub>13</sub> H <sub>11</sub> O <sup>+</sup>	183.0804	183.0810	3.09
175	C <sub>10</sub> H <sub>7</sub> O <sub>3</sub> <sup>+</sup>	175.0390	175.0394	2.62
167	C <sub>13</sub> H <sub>11</sub> <sup>+</sup>	167.0855	167.0859	2.02

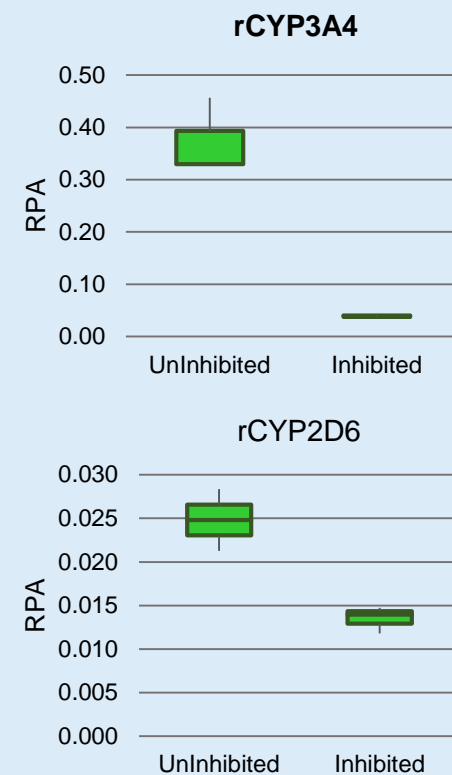




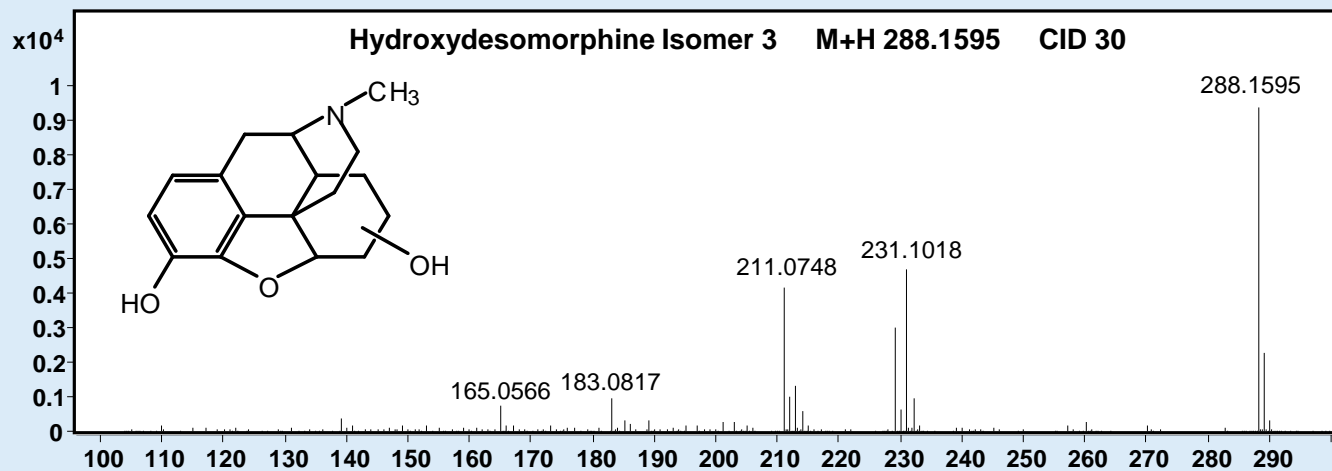
# Hydroxydesomorphine Isomer 2 MS<sup>2</sup>



M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	C <sub>17</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>	288.1594	288.1591	1.07
270	C <sub>17</sub> H <sub>20</sub> NO <sub>2</sub> <sup>+</sup>	270.1489	270.1515	9.88
231	C <sub>14</sub> H <sub>15</sub> O <sub>3</sub> <sup>+</sup>	231.1016	231.1010	2.33
213	C <sub>14</sub> H <sub>13</sub> O <sub>2</sub> <sup>+</sup>	213.0910	213.0915	2.53
195	C <sub>14</sub> H <sub>11</sub> O <sup>+</sup>	195.0804	195.0800	2.10
183	C <sub>13</sub> H <sub>11</sub> O <sup>+</sup>	183.0804	183.0768	19.69
175	C <sub>10</sub> H <sub>7</sub> O <sub>3</sub> <sup>+</sup>	175.0390	175.0383	3.72
167	C <sub>13</sub> H <sub>11</sub> <sup>+</sup>	167.0855	167.0856	0.59

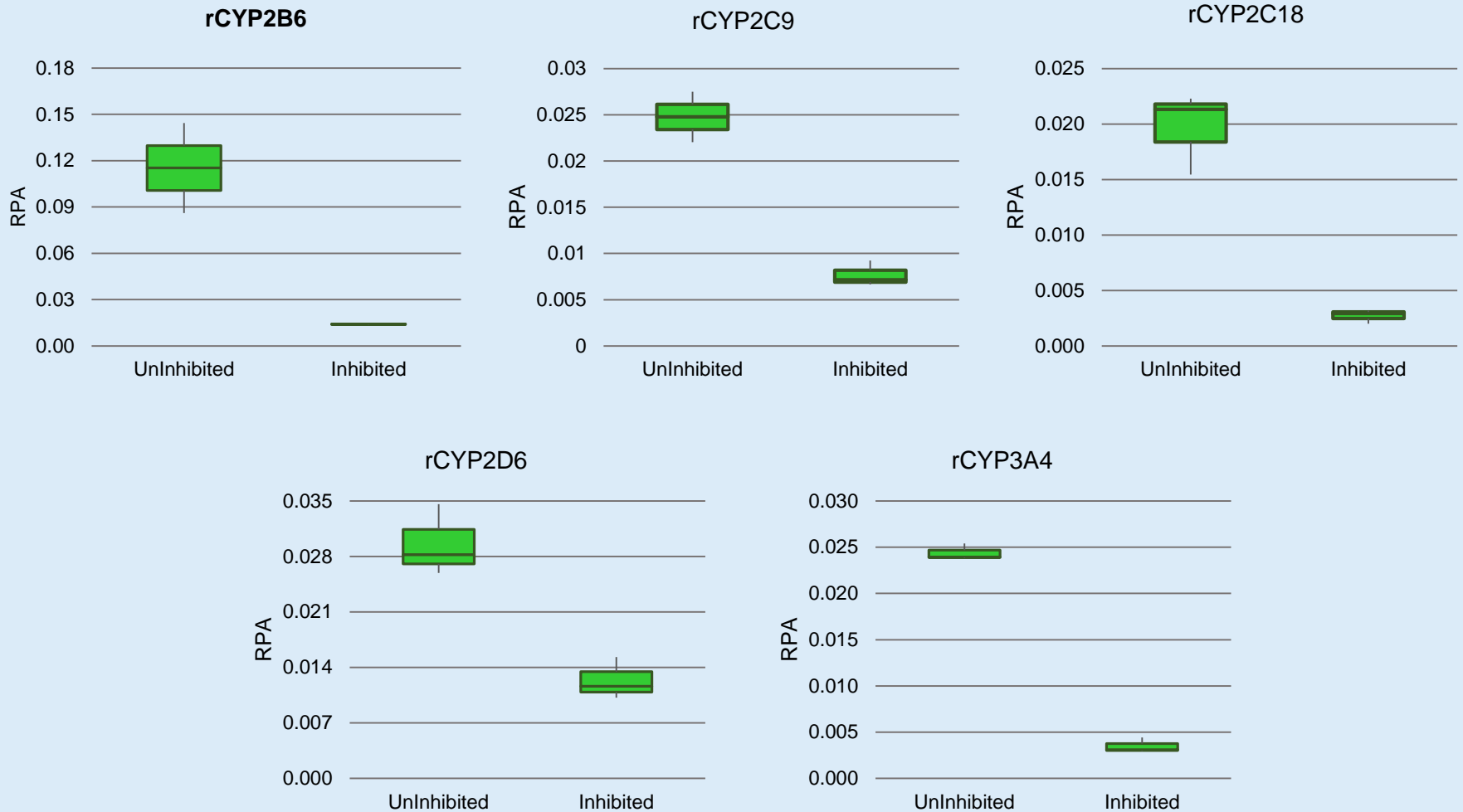


# Hydroxydesomorphine Isomer 3 MS<sup>2</sup>

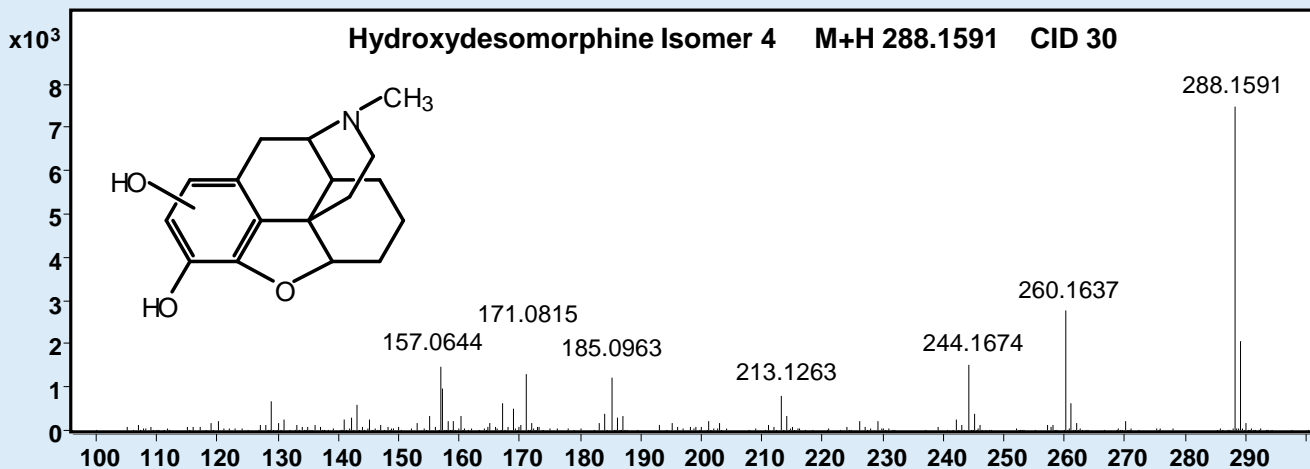


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	C <sub>17</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>	288.1594	288.1595	0.38
231	C <sub>14</sub> H <sub>15</sub> O <sub>3</sub> <sup>+</sup>	231.1016	231.1018	0.92
211	C <sub>14</sub> H <sub>11</sub> O <sub>2</sub> <sup>+</sup>	211.0754	211.0748	2.67
183	C <sub>13</sub> H <sub>11</sub> O <sup>+</sup>	183.0804	183.0817	7.09
165	C <sub>9</sub> H <sub>9</sub> O <sub>3</sub> <sup>+</sup>	165.0566	165.0583	0.99

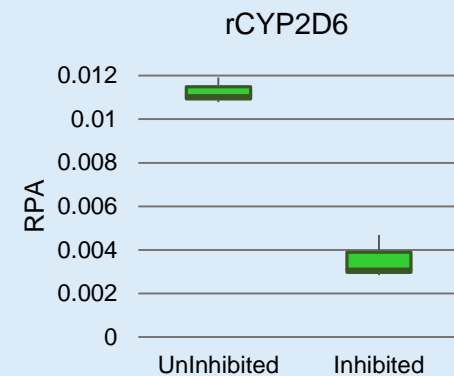
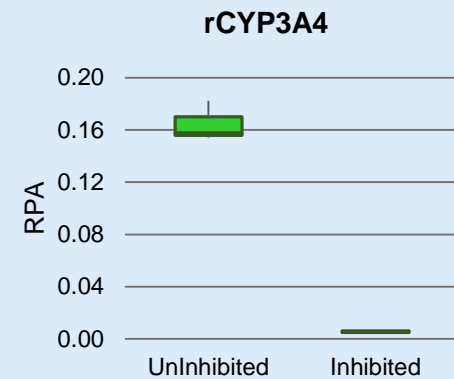
# Hydroxydesomorphine Isomer 3



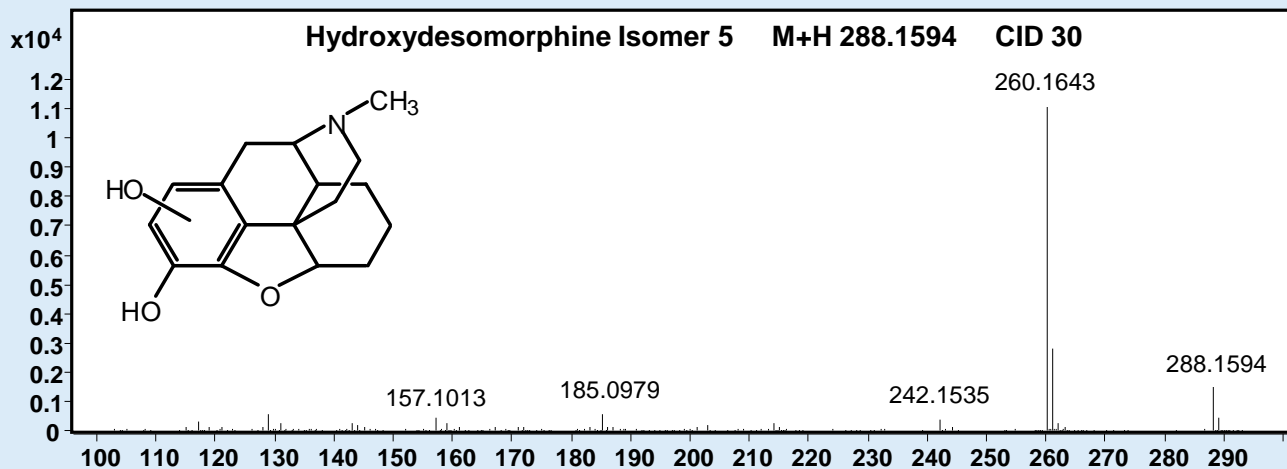
# Hydroxydesomorphine Isomer 4 MS<sup>2</sup>



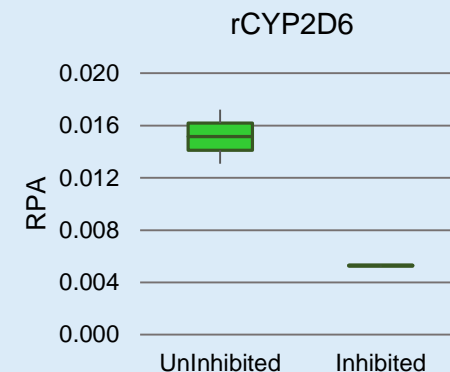
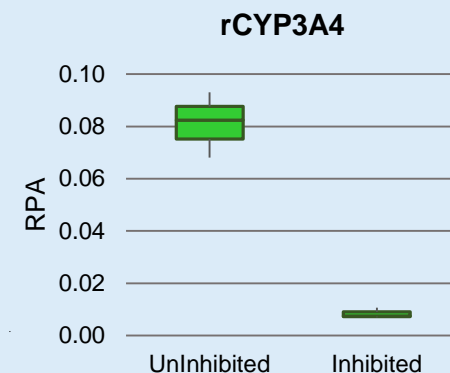
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	C <sub>17</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>	288.1594	288.1591	1.20
260	C <sub>16</sub> H <sub>22</sub> NO <sub>2</sub> <sup>+</sup>	260.1645	260.1637	2.92
244	C <sub>16</sub> H <sub>22</sub> NO <sup>+</sup>	244.1696	244.1674	8.92
213	C <sub>15</sub> H <sub>17</sub> O <sup>+</sup>	213.1274	213.1263	5.19
185	C <sub>13</sub> H <sub>13</sub> O <sup>+</sup>	185.0961	185.0963	1.19
171	C <sub>12</sub> H <sub>11</sub> O <sup>+</sup>	171.0804	171.0815	6.45
157	C <sub>11</sub> H <sub>9</sub> O <sup>+</sup>	157.0648	157.0644	2.69



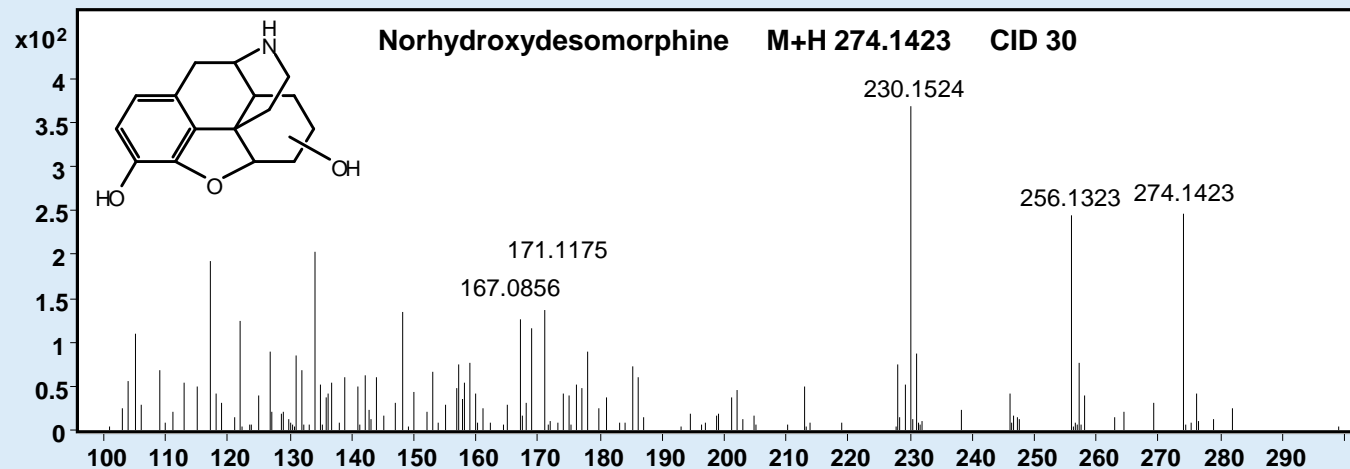
# Hydroxydesomorphine Isomer 5 MS<sup>2</sup>



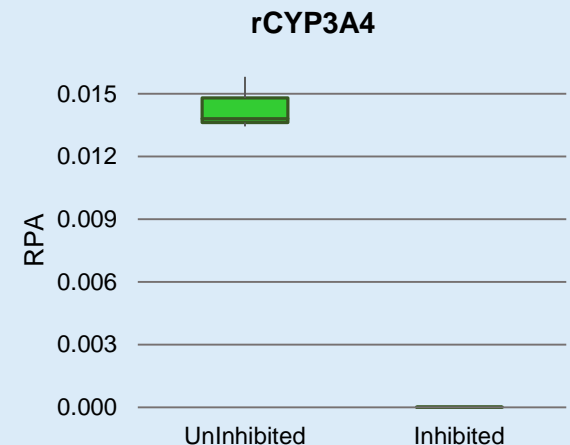
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	C <sub>17</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>	288.1594	288.1594	0.03
260	C <sub>16</sub> H <sub>22</sub> NO <sub>2</sub> <sup>+</sup>	260.1645	260.1643	0.62
242	C <sub>16</sub> H <sub>20</sub> NO <sup>+</sup>	242.1539	242.1535	1.95
185	C <sub>13</sub> H <sub>13</sub> O <sup>+</sup>	185.0961	185.0979	9.85
157	C <sub>12</sub> H <sub>13</sub> <sup>+</sup>	157.1012	157.1013	0.98



# Norhydroxydesomorphine MS<sup>2</sup>

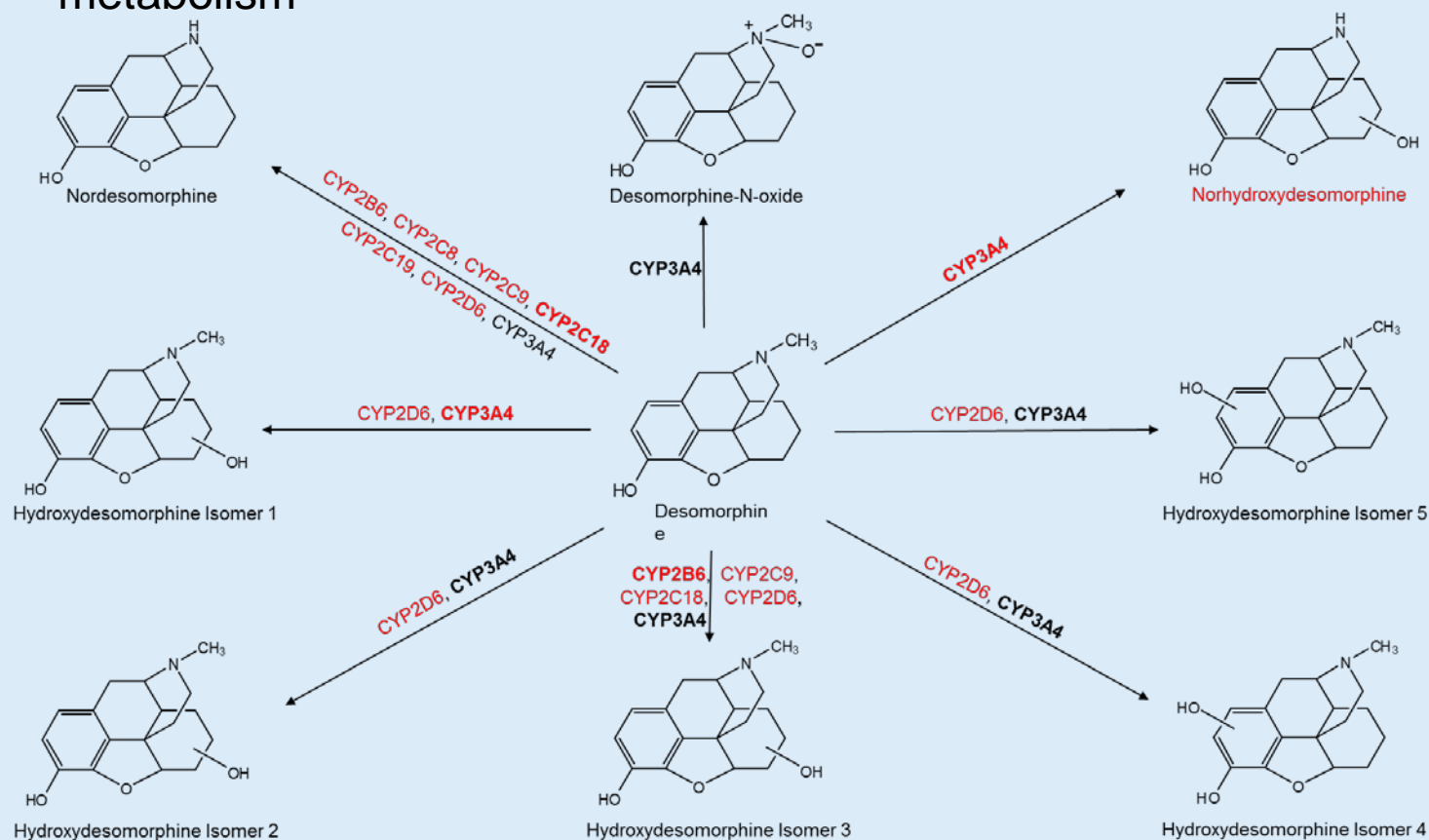


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
274	C <sub>16</sub> H <sub>20</sub> NO <sub>3</sub> <sup>+</sup>	274.1438	274.1423	5.21
256	C <sub>16</sub> H <sub>18</sub> NO <sub>2</sub> <sup>+</sup>	256.1332	256.1323	3.45
230	C <sub>15</sub> H <sub>20</sub> NO <sup>+</sup>	230.1539	230.1524	6.83
171	C <sub>13</sub> H <sub>15</sub> <sup>+</sup>	171.1168	171.1175	3.97
167	C <sub>13</sub> H <sub>11</sub> <sup>+</sup>	167.0855	167.0856	0.29



# Conclusions

- This study:
  - Identified a new Phase I metabolite of desomorphine
  - Suggests additional CYP isozymes may contribute to desomorphine's metabolism



# Questions?

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