



## LC-MS/MS Method for 89 Banned or Controlled Drugs in the Horse Racing Industry

UCT Part Numbers:

**SLDA100ID21-3UM** – Selectra<sup>®</sup> DA HPLC Column 100 x 2.1 mm, 3µm

**SLDAGDC21-3UM** – Selectra<sup>®</sup> DA Guard Cartridge Column 10 x 2.0 mm, 3µm

**SLGRDHLDR** - Guard Column Holder

The world of equine drug testing is constantly evolving. New regulations regarding banned and controlled substances can be found throughout North America, Europe and Australia [1,2]. The need for a reliable LC-MS/MS method for the determination and separation of a wide variety of drug classes is critical. In addition to traditional drugs of abuse, this equine testing panel also encompasses drug classes such as bronchodilators, diuretics, antihistamines and non-steroidal anti-inflammatory medications. The unique polyaromatic phase of the Selectra<sup>®</sup> DA HPLC column allows for efficient analysis and baseline separation of multiple drug classes at once, including several complex isobaric compounds.

### Instrumentation Parameters:

| HPLC Conditions   |   |                               |
|---|---|-------------------------------|
| <b>HPLC:</b> Agilent 1200 Series  |   |                               |
| <b>Column:</b> UCT, Selectra <sup>®</sup> , DA, 100 x 2.1 mm, 3 µm      |   |                               |
| <b>Guard column:</b> UCT, Selectra <sup>®</sup> , DA, 10 x 2.0 mm, 3 µm |   |                               |
| <b>Column temperature:</b> 40 °C  |   |                               |
| <b>Column flow rate:</b> 0.300 mL/min                                   |   |                               |
| <b>Auto-sampler temperature:</b> 10 °C                                  |   |                               |
| <b>Injection volume:</b> 10 µL  |   |                               |
| <b>Gradient program:</b>  |   |                               |
| Time (min)  | A% (0.1% formic acid in H <sub>2</sub> O) | B% (0.1% formic acid in MeOH) |
| 0   | 95  | 5                             |
| 5   | 40  | 60                            |
| 10  | 40  | 60                            |
| 10.1  | 0   | 100                           |
| 16.5  | 0   | 100                           |
| 17  | 95  | 5                             |
| 22  | 95  | 5                             |

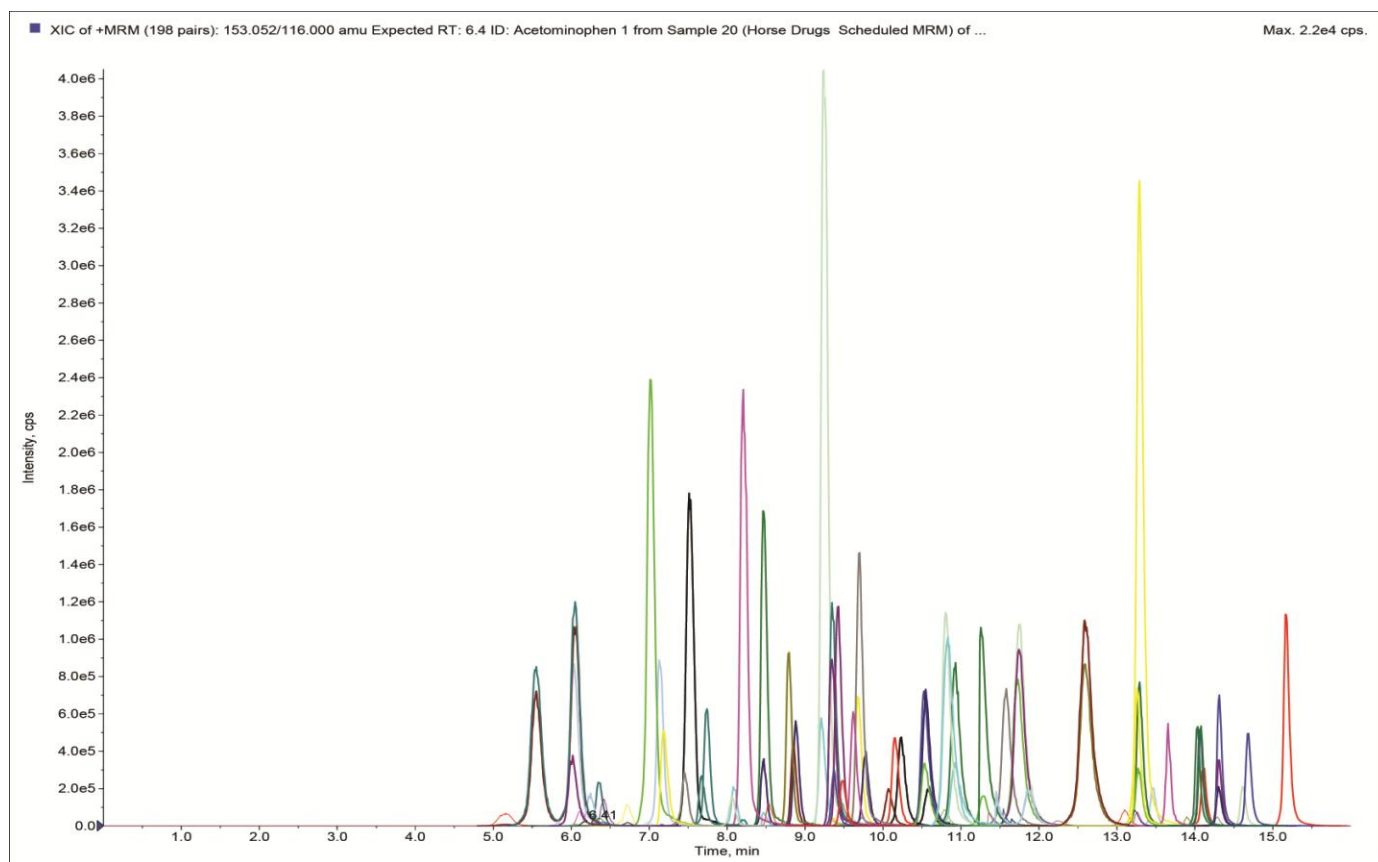
| MS parameters         |                      |
|-----------------------|----------------------|
| Instrumentation       | AB Sciex 4000 Q Trap |
| Polarity              | ESI +                |
| Spray voltage         | 5000 V               |
| Vaporizer temperature | 650 °C               |
| Collision gas         | Medium               |
| Cycle time            | 6.2 sec              |
| Acquisition method    | Scheduled MRM        |

| SRM Transitions  |                      |               |               |      |               |      |        |
|------------------|----------------------|---------------|---------------|------|---------------|------|--------|
| Analyte          | t <sub>R</sub> (min) | Precursor ion | Product ion 1 | CE 1 | Product ion 2 | CE 2 | DP (V) |
| Acetaminophen    | 6.4                  | 153.0         | 116.0         | 29   | 115.1         | 43   | 96     |
| Albuterol        | 5.3                  | 241.0         | 149.1         | 25   | 223.1         | 15   | 41     |
| Alprazolam       | 13.0                 | 308.9         | 205.3         | 17   | 281.2         | 17   | 31     |
| Amitriptyline    | 11.9                 | 278.2         | 91.1          | 17   | 105.1         | 17   | 46     |
| Amphetamine      | 6.0                  | 136.1         | 91.2          | 23   | 119.1         | 13   | 51     |
| Aripiprazole     | 13.0                 | 448.1         | 277.9         | 17   | 278.1         | 17   | 31     |
| Atenolol         | 6.3                  | 267.1         | 145.0         | 35   | 190.0         | 25   | 61     |
| Baclofen         | 6.4                  | 214.1         | 150.9         | 23   | 196.9         | 17   | 36     |
| Benzoyllecognine | 9.2                  | 209.1         | 168.0         | 25   | 105.1         | 39   | 76     |
| Benzocaine       | 9.6                  | 166.2         | 138.0         | 15   | 120.0         | 23   | 31     |
| Bupivacaine      | 9.1                  | 289.0         | 140.3         | 35   | 84.1          | 59   | 51     |
| Buprenorphine    | 10.6                 | 468.4         | 55.1          | 87   | 83.2          | 87   | 81     |
| Butorphanol      | 9.7                  | 329.2         | 311.2         | 33   | 158.2         | 61   | 71     |
| Caffeine         | 10.0                 | 195.0         | 138.0         | 27   | 110.0         | 33   | 36     |
| Carprofen        | 13.8                 | 274.2         | 228.0         | 15   | 193.2         | 43   | 36     |
| Chlordiazepoxide | 10.2                 | 300.0         | 227.1         | 21   | 283.0         | 35   | 56     |
| Chlorothiazide   | 14.2                 | 295.9         | 215.2         | 29   | 250.0         | 19   | 31     |
| Citalopram       | 10.0                 | 325.0         | 109.1         | 39   | 262.0         | 27   | 46     |
| Clenbuterol      | 8.3                  | 277.1         | 202.9         | 25   | 259.0         | 15   | 66     |
| Clidinium        | 9.7                  | 352.2         | 142.2         | 45   | 96.0          | 75   | 66     |
| Clonazepam       | 13.4                 | 316.1         | 270.2         | 39   | 241.2         | 37   | 56     |
| Clonzapine       | 9.5                  | 327.0         | 270.0         | 35   | 192.0         | 63   | 56     |
| Cocaine          | 9.2                  | 304.1         | 182.0         | 30   | 95.1          | 73   | 46     |
| Cocaine D3       | 9.2                  | 307.1         | 185.1         | 23   | 85.0          | 51   | 91     |
| Cyclothiazide    | 10.7                 | 390.1         | 317.2         | 33   | 100.0         | 49   | 41     |
| Dextrophan       | 8.6                  | 258.1         | 157.1         | 53   | 199.1         | 37   | 91     |
| Diazepam         | 14.5                 | 285.1         | 193.2         | 43   | 154.1         | 37   | 56     |
| Dothiepin        | 11.1                 | 296.1         | 251.1         | 25   | 225.1         | 25   | 61     |
| Edrophonium      | 8.3                  | 203.0         | 168.0         | 25   | 132.0         | 25   | 96     |
| Ephedrine        | 5.5                  | 166.2         | 148.0         | 11   | 117.0         | 25   | 36     |
| Estazolam        | 12.5                 | 295.3         | 100.2         | 31   | 208.1         | 31   | 61     |
| Fentanyl         | 10.5                 | 338.3         | 189.1         | 29   | 105.0         | 57   | 71     |
| Fentanyl D5      | 10.5                 | 342.2         | 188.3         | 39   | 105.0         | 53   | 51     |
| Flunitrazepam    | 14.1                 | 313.9         | 268.2         | 33   | 239.2         | 45   | 36     |
| Flurazepam       | 10.9                 | 388.1         | 315.1         | 37   | 134.1         | 67   | 46     |

|                       |      |       |       |    |       |     |     |
|-----------------------|------|-------|-------|----|-------|-----|-----|
| Furosemide            | 14.2 | 332.1 | 91.1  | 57 | 119.0 | 33  | 66  |
| Gabapentin            | 6.2  | 172.0 | 154.0 | 15 | 137.0 | 25  | 61  |
| Gabapentin D10        | 6.1  | 182.1 | 164.1 | 15 | 147.3 | 29  | 51  |
| Hexobarbital          | 10.7 | 273.0 | 157.0 | 17 | 81.0  | 29  | 41  |
| Hydrochlorothiazide   | 14.3 | 297.9 | 252.2 | 19 | 217.1 | 29  | 36  |
| Hydrocodone           | 8.0  | 300.0 | 199.0 | 39 | 128.0 | 50  | 46  |
| Hydromorphone         | 6.7  | 286.0 | 185.0 | 41 | 157.0 | 50  | 46  |
| Imipramine            | 11.7 | 280.9 | 85.9  | 25 | 58.1  | 63  | 61  |
| Imipramine D3         | 11.7 | 284.0 | 61.2  | 49 | 208.2 | 35  | 41  |
| Ketamine              | 8.8  | 238.1 | 125.0 | 30 | 220.0 | 21  | 36  |
| Ketamine D4           | 8.8  | 242.2 | 129.1 | 41 | 224.3 | 27  | 66  |
| Levorphanol           | 8.7  | 258.1 | 157.2 | 59 | 199.1 | 35  | 61  |
| Lidocaine             | 7.4  | 235.1 | 86.0  | 27 | 58.1  | 55  | 36  |
| Loratadine            | 14.9 | 383.1 | 337.2 | 31 | 267.2 | 45  | 76  |
| Lorazepam             | 13.1 | 321.0 | 303.5 | 21 | 275.0 | 29  | 46  |
| Lormetazepam          | 14.0 | 334.9 | 289.1 | 31 | 317.2 | 17  | 76  |
| Maprotiline           | 11.8 | 278.1 | 250.1 | 27 | 117.0 | 33  | 41  |
| MDA                   | 7.1  | 180.2 | 163.1 | 15 | 105.0 | 31  | 46  |
| MDEA                  | 8.3  | 208.1 | 163.0 | 19 | 105.1 | 37  | 41  |
| MDMA                  | 7.7  | 194.2 | 163.1 | 19 | 105.1 | 33  | 76  |
| Mepenzolate           | 9.5  | 340.2 | 130.0 | 41 | 77.0  | 101 | 46  |
| Meprobamate           | 11.3 | 219.0 | 191.0 | 31 | 178.0 | 31  | 126 |
| Methadone             | 13.2 | 310.1 | 265.0 | 19 | 105.0 | 35  | 56  |
| Methamphetamine       | 7.0  | 150.0 | 91.1  | 27 | 119.1 | 15  | 46  |
| Methamphetamine D5    | 7.0  | 155.2 | 92.1  | 25 | 121.2 | 15  | 26  |
| Mianserin             | 10.3 | 265.0 | 208.0 | 31 | 91.0  | 61  | 66  |
| Midazolam             | 10.8 | 326.0 | 291.0 | 50 | 222.0 | 50  | 60  |
| Mirtazepine           | 9.4  | 266.1 | 72.0  | 27 | 195.1 | 61  | 61  |
| Morphine              | 6.2  | 286.3 | 152.0 | 79 | 165.0 | 50  | 46  |
| Nalbuphine            | 8.4  | 359.2 | 341.1 | 31 | 255.0 | 43  | 71  |
| Nalorphine            | 13.0 | 311.3 | 266.1 | 25 | 105.1 | 41  | 71  |
| Naloxone              | 7.5  | 328.0 | 310.0 | 27 | 211.9 | 55  | 46  |
| Naltrexone            | 14.3 | 343.0 | 308.0 | 35 | 315.3 | 35  | 46  |
| Naproxen              | 13.7 | 231.1 | 185.0 | 21 | 170.2 | 37  | 36  |
| n-Butylscopolammonium | 9.2  | 360.1 | 130.1 | 59 | 194.1 | 31  | 51  |
| Neostigmine           | 7.5  | 224.1 | 72.0  | 49 | 209.3 | 29  | 66  |
| Nitrazepam            | 11.7 | 281.1 | 86.0  | 31 | 208.1 | 31  | 36  |
| Nortriptyline         | 11.7 | 264.1 | 190.9 | 33 | 105.1 | 33  | 51  |
| Olonzapine            | 7.1  | 313.0 | 256.0 | 33 | 84.0  | 35  | 66  |
| Orphenadrine          | 10.5 | 270.1 | 166.0 | 37 | 165.1 | 63  | 26  |
| Oxazepam              | 13.2 | 287.0 | 241.3 | 31 | 104.2 | 21  | 46  |
| Oxazeam D5            | 13.2 | 292.1 | 274.0 | 19 | 246.1 | 33  | 66  |
| Oxycodone             | 7.9  | 316.0 | 140.0 | 39 | 256.0 | 50  | 31  |
| Oxymorphone           | 6.3  | 302.0 | 227.0 | 37 | 198.0 | 50  | 36  |
| Paroxetine D6         | 12.3 | 336.1 | 76.2  | 53 | 198.1 | 25  | 81  |
| Paroxetine            | 12.4 | 330.0 | 192.0 | 27 | 123.0 | 37  | 61  |
| Prazepam              | 15.1 | 325.1 | 271.0 | 29 | 140.0 | 57  | 56  |
| Promethazine          | 11.2 | 285.1 | 86.0  | 29 | 71.1  | 61  | 41  |
| Propranolol           | 9.6  | 261.1 | 116.1 | 27 | 184.1 | 25  | 66  |
| Protriptyline D5      | 11.5 | 268.1 | 192.1 | 35 | 156.1 | 35  | 16  |

|                    |      |       |       |    |       |    |     |
|--------------------|------|-------|-------|----|-------|----|-----|
| Protriptyline      | 11.5 | 264.2 | 191.1 | 29 | 91.2  | 29 | 51  |
| Pseudoephedrine D3 | 6.0  | 169.1 | 151.1 | 15 | 117.0 | 29 | 56  |
| Pseudoephedrine    | 6.0  | 166.2 | 148.0 | 11 | 117.0 | 25 | 36  |
| Risperidone        | 10.6 | 411.1 | 191.1 | 39 | 69.0  | 81 | 61  |
| Sertraline         | 12.9 | 305.9 | 274.9 | 17 | 159.1 | 33 | 31  |
| Theophylline       | 8.2  | 180.9 | 123.9 | 27 | 95.9  | 33 | 31  |
| Trazodone          | 10.3 | 372.1 | 148.1 | 47 | 95.9  | 85 | 56  |
| Triazolam          | 14.3 | 343.0 | 308.1 | 39 | 315.0 | 37 | 81  |
| Trimipramine       | 12.4 | 295.1 | 100.2 | 51 | 58.1  | 51 | 51  |
| Vecuronium         | 9.2  | 557.4 | 100.0 | 71 | 356.4 | 55 | 121 |
| Venlafaxine        | 11.9 | 278.2 | 233.1 | 25 | 105.2 | 29 | 31  |
| Warfarin           | 14.0 | 308.9 | 163.0 | 19 | 251.1 | 27 | 51  |
| Zolpidem           | 14.0 | 309.0 | 163.0 | 23 | 251.0 | 49 | 61  |
| Zopiclone          | 9.3  | 388.9 | 244.9 | 19 | 217.1 | 27 | 46  |

## Chromatogram:



## REFERENCES:

1. [http://www.fei.org/sites/default/files/FEI\\_Prohibited\\_Substances\\_List\\_and\\_DB.pdf](http://www.fei.org/sites/default/files/FEI_Prohibited_Substances_List_and_DB.pdf)
2. <http://arcicom.businesscatalyst.com/model-rules---standards.html>

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