**Supplementary Materials**

**Table S1:** MS-instrumentation settings for lidocaine analysis. ESI, electrospray ionization; MRM, multiple reaction monitoring; amu, atomic mass unit; MW, molecular weight; IS, internal standard; GS, Gas sources; CAD, collisionally activated dissociation

|  |  |  |
| --- | --- | --- |
| Parameter | Adjusted value | |
| Interface | Turbo ion spray, ESI | |
| Ionization mode | Positive mode | |
| Detection mode | MRM | |
| Analyte | lidocaine | lidocaine-d10(IS) |
| MS Transitions | 235.1  86.1 | 245.1 96.1 |
| Dwell time (msec) | 200 | 200 |
| MW (amu) | 235 | 245 |
| DP (v) | 41.1 | 41.1 |
| EP (v) | 10 | 10 |
| CXP (v) | 29.3 | 29.3 |
| CE (v) | 14.6 | 14.6 |
|  | lidocaine / lidocaine-d10 (IS) | |
| Voltage (v) | 3000 | |
| Temperature (oC) | 550 | |
| GS1 (psi) | 60 | |
| GS2 (psi) | 60 | |
| CUR (psi) | 25 | |
| CAD | medium | |
| Data acquisition time | 2      minutes | |

**Table S2.** Lidocaine calibration standard solutions by adding 0.1ml of each working standard into 0.2 ml blank human serum.

|  |  |  |  |
| --- | --- | --- | --- |
| Calibration Standard Name | Spiked Working Standard Volume (ml) | Blank Human Serum Volume (ml) | Lidocaine Concentration (ng/ml) |
| STD 1 | 0.1 | 0.2 | 1000 |
| STD 2 | 0.1 | 0.2 | 800 |
| STD 3 | 0.1 | 0.2 | 600 |
| STD 4 | 0.1 | 0.2 | 400 |
| STD 5 | 0.1 | 0.2 | 200 |
| STD 6 | 0.1 | 0.2 | 100 |
| STD 7 | 0.1 | 0.2 | 50 |
| STD 8 | 0.1 | 0.2 | 25 |
| STD 9 | 0.1 | 0.2 | 12.5 |
| STD 10 | 0.1 | 0.2 | 6.25 |
| STD 11 | 0.1 | 0.2 | 3.13 |
| STD 12 | 0.1 | 0.2 | 1.56 |

**Table S3.** Lidocaine QC samples at three low, middle and high concentration levels. QCs were prepared by adding certain volumes of QC stock solutions and making up the volume to 10 ml by blank human serum.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | QC Stock Solution Concentration (µg/ml) | Volume of QC Stock (µl) | Final Volume (ml) | Final Lidocaine Concentration (ng/ml) |
| Low QC (LQC) | 0.4 | 100 µL of LQC Stock | 10 | 4 |
| Middle QC (MQC) | 10 | 150 µL of MQC Stock | 10 | 150 |
| High QC (HQC) | 50 | 100 µL of HQC Stock | 10 | 500 |

**Table S4:** Stability analysis of lidocaine calibration standards by LC-MS/MS. Three separate sets of experiments were performed to evaluate the stability of standard samples. The concentration range of Calibration standards was 1.54-1000 ng/mL.

|  |  |  |  |
| --- | --- | --- | --- |
| Calibrator nominal concentration (ng/mL) | Calibrator measured concentration (ng/mL) | RSD (%) | Accuracy (%) |
| 1.56 | 1.64±0.08 | 4.63 | 100.67 |
| 3.13 | 3.18±0.05 | 1.44 | 97.20 |
| 6.25 | 6.21±0.01 | 0.17 | 99.93 |
| 12.50 | 12.56±0.21 | 1.71 | 103.33 |
| 25.00 | 25.63±0.55 | 2.15 | 102.67 |
| 50.00 | 50.92±0.82 | 1.62 | 102.33 |
| 100.00 | 100.42±1.39 | 1.38 | 104.00 |
| 200.00 | 202.44±0.96 | 0.48 | 100.93 |
| 400.00 | 401.56±1.71 | 0.43 | 99.77 |
| 600.00 | 608.67±1.53 | 0.25 | 96.87 |
| 80.00 | 787.78±3.34 | 0.42 | 96.37 |
| 1000.00 | 1001.11±8.04 | 0.80 | 96.00 |

**Table S5: System suitability results of UPLC method validation for lidocaine estimation.**

|  |  |
| --- | --- |
| **System Suitability** | **Peak Area 125 μg/mL Lidocaine RS** |
| Sys\_Suitability\_1 | 80250 |
| Sys\_Suitability\_2 | 80306 |
| Sys\_Suitability\_3 | 80235 |
| Sys\_Suitability\_4 | 80214 |
| Sys\_Suitability\_5 | 80170 |
| Sys\_Suitability\_6 | 80250 |
| **Average** | 80237.5 |
| Std Dev | 45.00 |
| %RSD | 0.06 |

**Table S6:** Linearity range results of UPLC method validation for lidocaine estimation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Lidocaine**  **Conc. (μg/ml)** | **Area** | | | **Mean Area** |
| **1** | **2** | **3** |
| 0.7875 | 481 | 480 | 485 | 482.00 |
| 7.875 | 4661 | 4661 | 4621 | 4647.67 |
| 15.75 | 9377 | 9399 | 9414 | 9396.67 |
| 31.5 | 19219 | 19217 | 19201 | 19212.33 |
| 63 | 39533 | 39520 | 39551 | 39534.67 |
| 126 | 80109 | 80144 | 80166 | 80139.67 |
| 252 | 160102 | 159920 | 159994 | 160005.30 |

**Figure 1S**: The standard linear curve showed a linear response with a high linear correlation coefficient of 0.9998

**Table S7:** Accuracy results of UPLC method validation for lidocaine estimation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Lidocaine**  **Conc. (μg/ml)** | **Area** | | | **Mean Area** | **Std. Dev** | **%RSD** |
| **1** | **2** | **3** |
| 7.875 | 4655 | 4645 | 4626 | 4642 | 14.73 | 0.32 |
| 31.5 | 19281 | 19238 | 19336 | 19285 | 49.12 | 0.25 |
| 126 | 80088 | 80316 | 79141 | 79848.33 | 623.09 | 0.78 |

**Table S8:** Precision results of UPLC method validation for lidocaine estimation.

|  |  |
| --- | --- |
| **Lidocaine**  **Conc. (126 μg/ml)** | **Area** |
| Precision\_1 | 80193 |
| Precision\_2 | 80280 |
| Precision\_3 | 80277 |
| Precision\_4 | 79270 |
| Precision\_5 | 80033 |
| Precision\_6 | 79903 |
| **Average** | 79992.67 |
| Std Dev | 383.52 |
| %RSD | 0.48 |

**Table S9:** Stability results of UPLC method validation for lidocaine estimation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Lidocaine**  **126.00 (μg/ml)** | **Area** | | | **Mean Area** | **Mean cConc.\***  **(ug/ml)** | **Std. Dev** | **%RSD** |
| **1** | **2** | **3** |  |
| 0hr | 80109 | 80144 | 80166 | 80139.67 | 126.00 | - | - |
| 12hrs | 79951 | 80201 | 80275 | 80141.67 | 126.00 | 0.22 | 0.17 |
| 24hrs | 80106 | 80290 | 80399 | 80194.52 | 126.08 | 0.22 | 0.18 |
| 48hrs | 80030 | 79888 | 79920 | 80110.67 | 125.95 | 0.27 | 0.21 |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\*cConc.: Stands for calculated concentration based on the mean area of 0hr.

**Table S10:** Filter-binding results of UPLC method validation for lidocaine estimation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Lidocaine** | **Area** | | | | |
| **126.00 (μg/ml)** | **1 ml Saturation** | **2 ml Saturation** | **3 ml Saturation** | **4 ml Saturation** | **5 ml Saturation** |
| Filter binding\_1 | 80239 | 80344 | 80396 | 80403 | 80444 |
| Filter binding\_2 | 80201 | 80360 | 80493 | 80398 | 80339 |
| Filter binding\_3 | 80249 | 80351 | 80494 | 80459 | 80426 |
| **Average** | **80230** | **80352** | **80461** | **80420** | **80403** |
| Std Dev | 25.3 | 8.0 | 56.3 | 33.9 | 56.2 |
| %RSD | 0.03 | 0.01 | 0.07 | 0.04 | 0.07 |
| % Recovery | 100.1 | 100.3 | 100.4 | 100.3 | 100.3 |
| non-Filter Saturation\_126 | 80140 |  |  |  |  |