Automated calibration curve preparation and High Throughput analysis using LDTD-MS/MS system

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Introduction
Analytical laboratories have to prepare calibration curves for their sample analysis: large volumes of each concentration are made and transferred into smaller vials. Using a CERTUS FLEX liquid dispenser from GYGGER, an automated procedure for calibration curve preparation or aliquots is developed. Three solutions are used: a stock solution of the standard used, a dilution solution and a matrix solution. The system is set to a specific delivery volume for the preparation of the blank and 8 calibration standards directly in 2 mL vials.

After the calibration curve preparation, each blank and standards are extracted and analyzed using an LDTD®-MS/MS system.

CERTUS Flex Liquid Dispenser

LDTD-MS/MS System

Sample Preparation Method

Calibration curve preparation

Solution
Diluent: Mixture of Methanol:Water (75:25)
Stock solution: 10 µg/mL Cocaine in diluent solution
Matrix: Human urine.

Six calibration curves (One blank and 8 calibration points) are prepared per batch using a 54 positions rack, 2 mL vials and the CERTUS FLEX liquid dispenser.

Table 1 – Solution volume used

<table>
<thead>
<tr>
<th>Standard ID</th>
<th>Diluent (µL)</th>
<th>Stock (µL)</th>
<th>Matrix (µL)</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>100</td>
<td>0</td>
<td>900</td>
<td>0</td>
</tr>
<tr>
<td>STD 1</td>
<td>99</td>
<td>1</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>STD 2</td>
<td>98</td>
<td>2</td>
<td>900</td>
<td>20</td>
</tr>
<tr>
<td>STD 3</td>
<td>90</td>
<td>10</td>
<td>900</td>
<td>100</td>
</tr>
<tr>
<td>STD 4</td>
<td>80</td>
<td>20</td>
<td>900</td>
<td>200</td>
</tr>
<tr>
<td>STD 5</td>
<td>60</td>
<td>40</td>
<td>900</td>
<td>400</td>
</tr>
<tr>
<td>STD 6</td>
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<td>600</td>
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<td>STD 7</td>
<td>20</td>
<td>80</td>
<td>900</td>
<td>800</td>
</tr>
<tr>
<td>STD 8</td>
<td>0</td>
<td>100</td>
<td>900</td>
<td>1000</td>
</tr>
</tbody>
</table>

Extraction procedure

Liquid-Liquid extraction.
In a 1.5 mL Eppendorf tube, add:
- 100 µL of Phosphate buffer (0.1M, pH8)
- 10 µL internal standard solution (Cocaine-d3, 1 µg/mL)
- 100 µL urine standard solution
- Vortex 10 seconds
- 400 µL Hexane:Ethyl Acetate (1:1)
- Vortex 20 seconds
- Spot 4 µL of upper layer in a LazWell™ plate
- Evaporate to dryness
- LDTD-MS/MS analysis after complete solvent evaporation

Table 2 - MRM transitions

LDTD®-MS/MS Parameters

LDTD
Model: Phytronix, SH-960
Carrier gas: 3 L/min (air)
Laser pattern: 6 seconds ramp to 45% power.

MS/MS
Model: Shimadzu LCMS-8060
Dwell Time: 50 msec
Pause Time: 3 msec
Total run time: 9 seconds per sample
Ionization: APCI
Analysis Method:
- Positive MRM transition
Results and Discussion

CERTUS FLEX Method

The stock and dilution solution are dispensed in vials using a GYGER micro valve, number 21767 (0.15mm nozzle and 0.03mm travel). The matrix solution is first filtered on a Whatman paper P4 to remove large particles. The micro valve number 21770 (0.30mm nozzle and 0.1 mm travel) is used to transfer 900 µL urine in the vials. One blank and 8 standards are prepared in nine vials of 2 mL. With a 54 position rack, 6 curves can be prepared simultaneously during a batch. Figure 3 shows a typical micro valve used on the CERTUS flex system.

Figure 3 – Micro valve

LDTD®-MS/MS screening Method

Drugs are extracted using a Liquid-Liquid procedure and analyzed using a MRM method in positive mode. After a fast desorption, fortified and blank samples are evaluated. Using the peak area ratio, calibration curves are prepared and aliquoted in less than 2 minutes.

Linearity evaluation

Spiked samples are extracted, analyzed and the peak area ratio is used to evaluate the linearity of each calibration curve made with the CERTUS FLEX system. Slope and correlation coefficient of each curve were compared. Figure 4 shows the results for all calibration curves analyzed. In Table 3, slope value within 0.0069296 and 0.0071517 are reported showing good consistency between each preparation. Also, linearity coefficients greater than r² > 0.996 are showed.

Accuracy evaluation

All standards are used to build the calibration curve. Using the peak area ratio, the back calculated concentration of each standard is evaluated. Then, calculated values are compared to the theoretical value to get the nominal percentage (%Nom). In Table 4, the lowest and highest %Nom values are showed. Results within ±15% are reported.

Conclusion

The CERTUS FLEX liquid dispenser system allows the automatic preparation of six calibration curves (8 standards and 1 blank), using only 3 solutions (stock solution, dilution and matrix). Reproducibility, linearity and accuracy curves are prepared and aliquoted in less than 2 minutes.

The LDTD technology combined with a mass spectrometer system allows the ultra-fast (9 seconds per sample) quantification of Liquid-Liquid extracted curves.