A Paper-free Fully Regulated Bioanalytical Assay Validation of Fentanyl in Human Plasma

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Purpose

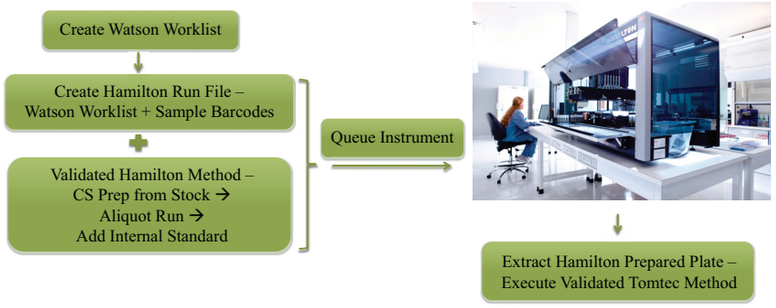
A fully validated bioanalytical method for fentanyl in EDTA human plasma spanning 5.00 - 5000 pg/mL was generated in a completely paperless laboratory. The model presented illustrates the application and interaction of multiple electronic laboratory systems to execute and document a validation study in a fully electronic, regulatory compliant environment.

Methods

Fentanyl and fentanyl-D5 were extracted via liquid-liquid extraction in MTBE under basic conditions. Instrumental analysis consisted of UPLC gradient separation on a Waters HSS T3 column with monitoring of m/z 337 to 188, and 342 to 188 transitions. Data were acquired directly into Watson LIMS via TSQ Module interface with a Thermo Vantage mass spectrometer.

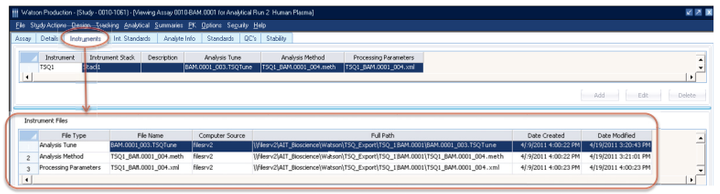
Prior to extraction, fresh, robotically prepared calibration standards (CS) were added to each extraction plate. All validation samples and internal standard were prepared and/or aliquotted using a Hamilton Microlab Star. Extraction occurred via Tomtec Quadra96.

Sample Preparation - Robotic run preparation workflow:



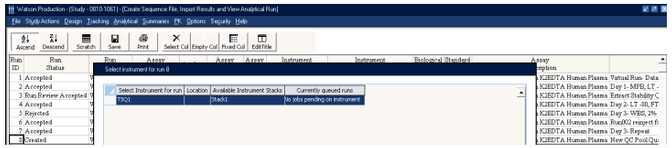
Instrumental Analysis – Data collection and processing workflow:

Import Instrument Method – Watson Run Assay – All necessary LC-MS parameters for full instrument control via Watson LIMS are imported into and stored within the Part 11 compliant Watson database.



Methods (Cont.)

Queue Instrument – Submit Runs to LC-MS instrument queue via Watson LIMS:



Acquire and Package Data – Active Instrument view within Watson TSQ Module during data acquisition:

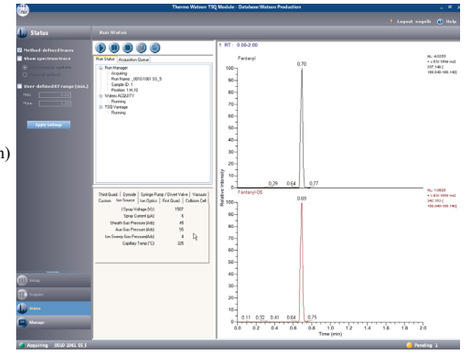
Live: 1.) Chromatograph

2.) MS Parameters Readback

3.) LC Parameters Readback (not shown)

4.) Mass Spectrum (not shown)

5.) Data Packaging → Automatic Transfer to Watson Database Following each Injection



Process Data – Peak integration & chromatographic review within Watson LIMS:

…Using any computer with Watson, process data acquired on any instrument, from any project, study, run:

1.) Integrate / Reintegrate

2.) Review – Area/Height, RT, Ratio, Inj. Vol., Vial Position, etc.

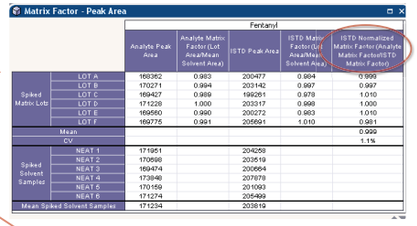
3.) ISTD – Plot Response vs. Injection, Calc. Mean / Stdev.

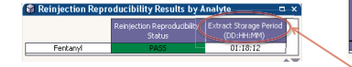
4.) Accept Integrations – ***Irreversible & Programatically Required Before Regression is Possible***

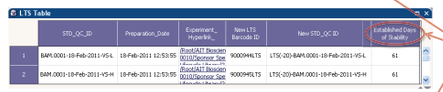
5.) Regress Any Analytes

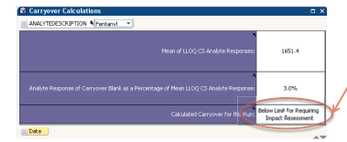
Data Analysis – Automatic data analysis & processing via customized templates in IDBS E-workbook:

1.) Matrix Factor, Recovery, Selectivity etc. Analysis, & Report Tables are Auto-calculated / Generated



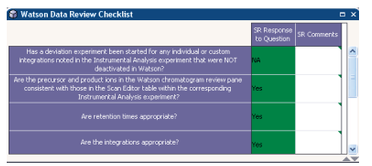






2.) Validated Stability Windows, Freeze/Thaw Cycles & Reinjection Reproducibility Storage Period are Calculated; Control Matrix & Carryover Acceptability per SOP are Determined

3.) Scientific and Quality Data Reviews – E-workbook (& Watson not shown)



Results

Inter-run (n=3) accuracy and precision were ≤3.2% and ≤ 5.7%, respectively. Carryover was ≤ 6.6% of the mean LLOQ CS response. Selectivity was ≤ 3.2% of the mean LLOQ CS. Spiked selectivity accuracy and precision were ≤ 0.8% and ≤ 1.3%, respectively. IS normalized matrix factor was 0.999. Consistent recovery of the analyte and IS were observed across the assay range. Hemolysis (2% lysed blood) demonstrated no significant impact.

Stabilities - Whole blood (2.25 hrs, wet ice); benchtop (47 hrs, ambient); freeze/thaw and long term (5 cycles and ≥61 days, -20 C and -80 C); reinjection reproducibility (42 hrs, 2-8 C).

All data recording, acquisition, analysis and reviews were executed within a part 11 compliant environment.

Conclusion

A network of electronic applications and laboratory instrumentation enabled efficient and comprehensive, paper-free execution and documentation of a fully regulated bioanalytical assay validation.