

Automated and Validated Bioanalytical LC-MS/MS Data Analysis

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Overview

- Bioanalytical projects require numerous types of calculations to be performed on LC-MS/MS data.
- Manually transferring MS/MS data into an external spreadsheet program to perform calculations requires in-depth secondary review.
- A more efficient workflow is presented here in which LC-MS/MS data is acquired directly into a LIMS system and automatically accessed by an electronic laboratory notebook for performing calculations.
- This validated workflow does not require, nor allow, manual data manipulation, simplifying quality review.

Introduction

Typical bioanalytical projects require that numerous types of calculations be performed with LC-MS/MS data. Examples include stock solution comparisons, system suitability analysis, scientific review of a run in Watson, working solution comparisons, and even tabulation of data for selectivity, matrix factor, and whole blood stability experiments. Most often, performing such calculations requires importing or copying and pasting LC-MS/MS data into a spreadsheet program. Any procedure dependent upon and/or allowing manual data manipulation is susceptible to at least the possibility of human error, and thus requires in-depth secondary review, especially for regulated work. Presented here is an automated and validated workflow in which LC-MS/MS data acquired by a LIMS system is directly accessed by an electronic laboratory notebook (ELN) for performing numerous types of calculations, all without requiring, or allowing, manual data manipulation. This approach is instantaneous, accurate every time, and eliminates the need for in-depth secondary review, reducing turnaround times for bioanalytical validations and other projects.

As an example, the workflow for performing a **stock solution stability evaluation** is presented here.

Methods

Bioanalytical LC-MS/MS data is acquired directly into the Watson LIMS database via the TSQ Module interface using a Thermo TSQ Vantage mass spectrometer. A customized, validated ELN (IDBS E-WorkBook Suite) template used for comparing stocks or assessing stock stability pulls the required information from Watson into the template using database links. Using pre-defined sample nomenclature in Watson, the data is sorted for each analyte and internal standard (IS) with the appropriate SOP acceptance criteria applied. The template automatically verifies sponsor and compound for each experiment and ensures that comparison solutions originate from intended stocks. It also confirms that the stock and comparison dilutions were prepared on the same date. Embedded logic calculates percent difference or percent change based on the user selecting comparison or stability, respectively. The stability time interval is calculated from solution preparation and sample injection time-date stamps.

Key Instrumentation and Software

- Thermo Scientific TSQ Vantage mass spectrometer
- Watson LIMS v 7.4 SP2 and TSQ Module v 1.0
- IDBS E-WorkBook 2010 v 8.3.1

Results

Figure 1. Scan the fresh and aged stock solutions to be compared

Stock_Solution ID	Fresh or Stock Solution 1	Aged or Stock Solution 2
Stock-Methadone- FE4444-01-000932	Stock-Methadone- FE4444-01-000946	
Substance Type	Small Molecule	Small Molecule
Substance Use	Analyte	Analyte
Substance Name	Methadone	Methadone
Sponsor Number of Stock	9999	9999
Substance Concentration	0.10	0.10
Substance Concentration Unit	mg/mL	mg/mL
Experiment Hyperlink	Root\AIT Bioscience\Item\Root\AIT Bioscience\Item Library\Approved Template Library\Approved Template Folder\Stock Solutions\Comparison_v2\Stock-Methadone-FE4444-01-000932	Root\AIT Bioscience\Item\Root\AIT Bioscience\Item Library\Approved Template Library\Approved Template Folder\Stock Solutions\Comparison_v2\Stock-Methadone-FE4444-01-000946
Solution Preparation Date	28-Jan-2011	24-Nov-2010
Time Interval (Days)	65	

Figure 2. In ELN, create dilutions of both stock solutions (with IS) and scan these into the experiment

	Fresh or Solution 1	Aged or Solution 2
Scan Solution(s)	SS-1-000779	SS-1-000780
Storage Date and Time	28-Jan-2011 8:23:17	28-Jan-2011 8:43:41
Preparation Date of SS	28-Jan-2011	28-Jan-2011
Expiration Date of SS	13-Apr-2011	13-Apr-2011
Experiment ID	9999-SS-000779	9999-SS-000780
Experiment Hyperlink	Root\AIT Bioscience\Template Lifecycle Library\Approved Template Test Records Folder\Stock Solutions\Comparison_v2\9999-SS-000779(v2)	Root\AIT Bioscience\Template Lifecycle Library\Approved Template Test Records Folder\Stock Solutions\Comparison_v2\9999-SS-000780(v2)

Figure 3. Enter Watson LIMS run designation

Run Information	Entry
Study Number	9999-2001
Run Number	2

While the math is quite simple, this process provides numerous significant features. The ELN template does not allow the user to change data or to inadvertently input incorrect data. The ELN uses a direct database link to access the Watson response data and at the same time electronically capture the exact time and date that various steps took place, thereby determining the exact length of time for which stability is determined. This information can be used subsequently to prevent the use of expired stock solutions based on the most current stability data.

Figure 5. ELN calculation of response ratios.

Test Response Ratio	Solution 1	Solution 2
Row 1	0.757025478	0.747043731
Row 2	0.757400119	0.756797442
Row 3	0.736886351	0.745375534
Row 4	0.745607805	0.758627488
Row 5	0.753207240	0.767036873

Figure 6. Status determined by embedded business logic using SOP criteria

Status Check - Stability Comparison	Entry
Average of Response Ratio-Fresh	0.7500254
Average of Response Ratio-Aged	0.7549762
Percent Change	0.7
Stability Status	Pass

Figure 4. Instantaneous query of Watson LIMS database to retrieve analyte and IS response

	Area			
	Analyte	IS	Analyte	IS
Row 1	1670028.92	2206040.58	1695573.12	2269710.66
Row 2	1723214.72	2275170.91	1756906.11	2321501.12
Row 3	1747365.62	2371282.38	1792164.77	2404378.31
Row 4	1806775.14	2423224.55	1862337.22	2454877.06
Row 5	1879117.12	2494820.84	1916908.55	2499108.74

Figure 7. Experiment summary

Comparison Summary	Entry
Study	9999-2001
Method	BAM_0002
Run ID	002
Substance Name	Methadone
Substance Type	Small Molecule
Substance Use	Analyte
Mean Ratio Solution 1	0.75002539835281
Mean Ratio Solution 2	0.75497619025508
Time Interval (Days)	65
Percent Difference or Change	0.7
Stability Status	Pass

Conclusions

Because the template and network database systems have been validated, there is no need for further secondary review of the stability or comparison data. Data processing is standardized, while the potential for human error is significantly decreased. During run preparation, fields requiring user entry in the template can be completed rapidly by barcode scanning or via dropdown menus within a few minutes. Following data acquisition, comparison results are obtained in seconds using a database link to the Watson data. The template then displays the final Pass or Fail status of the stock stability experiment entirely based on whether the data meet the business rules (SOPs) for acceptance criteria. While this is a general example of the process, similar workflows have been designed and validated to reduce human error and tabulate data for a report as needed for stability tests in matrix, carryover evaluations, selectivity, matrix factor, recovery, and specificity.