Quantification Reporting using Targeted Mass Spec Assays with Panorama and Skyline

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Panorama

http://panoramaweb.org/

Overview

Panorama's new reporting tools further extend its capabilities for managing absolute quantification results generated by targeted mass spectrometry assays. Panorama first added support for quantification assays in 2017, following closely on the Skyline targeted mass spec desktop application. This recent work reduces the number of external analysis tools required to analyze this data, as well the manual steps previously required to transfer data between applications. Additionally, Panorama's user interface offers improved navigation, and the expanding data model can be leveraged to generate interactive charts or via custom scripts in languages like R. Developed as a module in the LabKey Server web-based data management platform, Panorama is freely available, open source and may be installed and managed by any interested group.

Introduction

Acquire Raw Data Files

- Skyline, a Windows application, supports all major mass spec vendors file formats natively
- SkylineRunner provides a command-line interface to automate analyses

Configure Quantitation in Skyline

- Skyline captures information about samples and replicates
- Define peptides, small molecules, and transitions to monitor, and quantification settings, including standards, unknowns, blanks, and QCs

Upload to Panorama

- Skyline integrates with Panorama to easily transfer data and iterate on data analysis
- Panorama serves as a data repository and collaboration tool, and supports the full Skyline data model

Interactively explore, analyze, and export data

- Web-based interface is suitable for easily sharing data with non-Skyline users
- Create custom reports and visualizations using programming tools, or export data for external analysis

Methods

Skyline and Panorama combine to provide a workflow for managing and analyzing LCMS-based large and small molecule experiments. Absolute quantification is performed using either single-point or multiple-point calibration curves by spiking a heavy-labeled 'standard' analyte of known abundance. Users configure quantification within Skyline and upload their document to Panorama, which offers a growing library of reporting and analytics options. The underlying data is accessible via APIs and can be exported to Excel and other common formats.
All tools are released under the Apache 2.0 open source license.

Results

Figures of Merit

CONFIGURE QUANTIFICATION IN SKYLINE

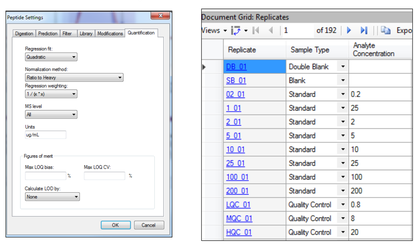


Figure 2: Calibration curves are defined within Skyline. Users select regression fit options, and configure which peptides or small molecules to use for fitting the curve. Samples and replicates define expected concentrations for analytes.

UPLOAD TO PANORAMA

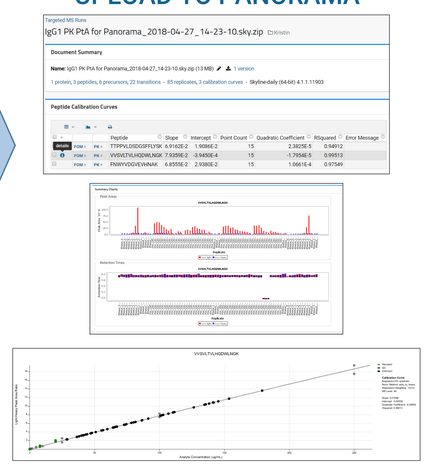


Figure 3: The user initiates an upload action from within Skyline to push the Skyline document to Panorama, which imports the data and makes it available through its web interface. Reports include summaries of each quantified peptide or small molecule, plots of intensity and retention times across samples, calibration curves, and tables of results.

VIEW FIGURES OF MERIT RESULTS

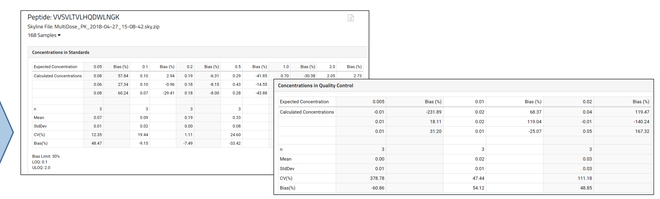


Figure 4: Panorama calculates figures of merit automatically, including coefficient of variation, bias, standard deviations, and limits of quantitation. The initial release, version 18.1, focused on comparability with Merck's existing Watson-based analysis, which defined a 30% bias limit. Future releases will support configurable bias and CV cutoff thresholds as recently introduced in Skyline-daily. Results can be exported to Excel.

Pharmacokinetics

SPECIFY SAMPLE PROPERTIES

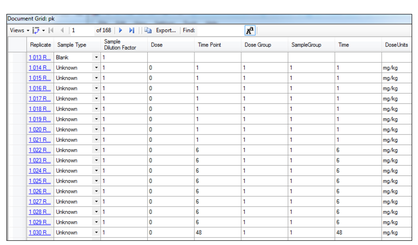


Figure 5: To perform PK calculations, Panorama needs additional properties for each sample, including its timepoint, initial dose, and grouping information to specify which samples are part of the same time course. They are configured in Skyline as replicate annotations.

VIEW PHARMACOKINETICS RESULTS

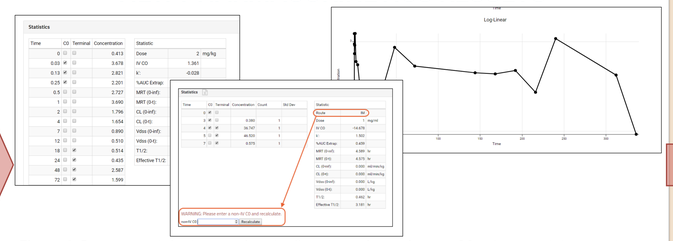


Figure 6: Panorama defaults to using the first three timepoints as C0 values, and the last three as Terminal values. Users can interactively adjust them, using time plots as reference. As the user adjusts the values, Panorama automatically recalculates and displays the updated results, and saves the configuration as the default for the next time the PK reports are shown. Version 18.1 handles IV PK calculations, and the upcoming version 18.2 adds support for non-IV uses, including IM. There are two separate PK datasets shown here, one IV and one IM.

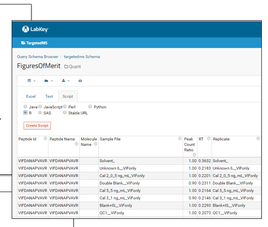
SEE ALSO

TP 732: Protein turnover experiments using Skyline
WP 072: Quantitative data independent acquisition analysis of Pompe disease biomarkers: a foundation for improved targeted method

LEVERAGE PANORAMA DATA

SQL

```
SELECT
  sub.PeptideId,
  sub.MoleculeId,
  sub.Time,
  sub.SubGroup,
  MAX(sub.Sequence) AS Peptide,
  MAX(sub.Filename) AS FileName,
  MAX(sub.ionName) AS ionName
FROM
  ...
```



R

```
library(Rlabkey)
labkey.data <- labkey.selectRows(
    baseUrl="https://localhost/labkey",
    folderPath="/TargetedMS/Quant",
    schemaName="targetedms",
    queryName="FiguresOfMerit")
```

Python

```
import labkey
server_context =
    labkey.utils.create_server_context(
        'localhost',
        'TargetedMS/Quant',
        'labkey', use_ssl=True)
my_results = labkey.query.select_rows(
    server_context=server_context,
    schema_name='targetedms',
    query_name='FiguresOfMerit')
```

Figure 7: Panorama provides robust APIs for a variety of popular programming languages that allow developers to access the underlying data, always accessed in the context of an authorized user. It also offers SQL access to the underlying data model. A partial SQL query is shown that is used to calculate quantitation reporting results. All data grids generate code snippets to use the LabKey APIs for extracting data. Example snippets for R and Python are also shown.

Conclusions

Panorama now offers a variety of calibrated quantification reporting. In addition to the previously supported calibration curves and data tables, Panorama now calculates figures of merit and pharmacokinetics (PK) analysis, including charts. Panorama piggybacks on data embedded in the Skyline files for context, including expected concentrations, timepoints for sample creation, and more. Users can interact with the data, choosing starting and terminal time points and saving the results. Panorama will add additional preconfigured reports, including pharmacodynamic modeling. All of the data in Panorama is accessible through APIs, allowing users to create scripts in R and other programming languages to enable additional reports. By adding in this functionality, users are able to streamline processes that were once cumbersome and spread out across different software platforms. The impact will help reduce the data processing bottleneck in many laboratories. As of May 2018, more than 300 labs are using Panorama for free to manage targeted mass spectrometry assays on http://panoramaweb.org/, a server hosted by the University of Washington. Additionally, major pharmaceutical companies and other organizations have deployed their own in-house installations of Panorama.

References

Sharma V, MacLean B, et al. J. Proteome Res. 2014/08; 10.1021/pr5006636.
Abbatiello S, Carr S et al. Mol & Cell Proteomics. 2015/02; mcp.M114.047050.
Sharma V, Eckels J et all. Mol & Cell Proteomics. 2018/02; 10.1074/mcp.RA117.000543

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