

Agilent MassHunter Easy Access Software for Lot-to-Lot Purification Analysis of a Model Therapeutic Protein

Application Note

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Abstract

This application note describes the use of Agilent Easy Access software to analyze lot-to-lot purity of a model therapeutic protein as performed by a bioanalytical scientist in a typical biopharmacology setting. The lot-to-lot purity of the model therapeutic protein was analyzed using an Agilent 1290 Infinity LC system coupled to an Agilent 6530 Accurate-Mass Q-TOF LC/MS with data analysis provided by Agilent Easy Access Software. The excellent chromatographic resolution and mass accuracy of the LC/MS platform, combined with powerful data processing capabilities, enabled easy and rapid analysis of protein purity across batches. This application simplifies the workflow and provides faster decision making for further downstream processing. The software is useful for comparing protein profiles for batch consistency, which is required by regulatory bodies. In addition, it helps with the understanding of the impurity profiles of purified proteins during scale-up or manufacture. This data will be invaluable for companies developing biosimilars, aiding in the assessment of their product's similarities to the original before market commercialization.



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Introduction

Proteins are recognized as biotherapeutic molecules whose important medical applications require them to undergo rigorous product testing prior to release. Therapeutic protein manufacturing procedures, although very well defined, may still lead to some product or process related impurities. Small adjustments to the manufacturing process such as pH, temperature, and compositional changes in culture media can affect the final product quality. The batch-to-batch variation in a manufacturing process can also lead to micro-heterogeneity in the product. Therefore, it is very important to analyze any change implemented in the manufacturing process. This application note describes the use of an LC coupled to a high definition Q-TOF to analyze lot-to-lot variations of a model therapeutic protein. Easy Access software allows personnel that are inexperienced with mass spectrometry to use the LC/MS system for increased productivity and efficiency. It also provides an easy and convenient method for analyzing protein purity, helping to determine the best way to further process the samples if necessary.

Experimental

Sample

Different lots of a model therapeutic protein were obtained from GangaGen Biotechnologies Pvt. Ltd, Bangalore, India.

Instrumentation

LC system

Agilent 1290 Infinity LC

MS system

Agilent 6530 Accurate-Mass Q-TOF LC/MS with Agilent JetStream Technology

Analytical software

MassHunter Easy Access Software (B.05.00)

The method parameters are listed in Table 1.

Table 1. Experimental parameters.

Parameter	Agilent 1290 LC
Column	Agilent Poroshell 300SB-C18, 2.1 × 75 mm, 5 μm (p/n 660750-902)
Column temperature	60 °C
Sample thermostat	5 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	90 % acetonitrile in water with 0.1 % formic acid
Gradient	0 minutes 20 % B 0–15 minutes 85 % B 15–16 minutes 20 % B
Stop time	16 minutes
Flow rate	0.5 mL/min
Ion mode	Positive ion mode, ESI (Profile)
Drying gas temperature	350 °C
Drying gas flow	8 L/min
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Nebulizer	35 psi
Capillary voltage	3,800 V
Nozzle	1,000 V
Data analysis	The data obtained from LC/MS were analyzed using Agilent MassHunter Qualitative Analysis software B.05 and Agilent MassHunter BioConfirm software B.05

Results and Discussion

Due to regulatory requirements of the biopharmaceutical industry, drug substances are routinely monitored for chemical consistency and purity. Sensitive analytical techniques are required to determine the effect that process changes have on the identity, strength, quality, purity, and potency of a drug, as they relate to the safety and effectiveness of the final product. Advancements in sensitive analytical techniques such as high performance liquid chromatography (HPLC), mass spectrometry (MS), nuclear magnetic resonance (NMR), and capillary electrophoresis have provided more accurate methods of evaluating the effect process changes may have on these substances. Figure 1 shows the workflow using Agilent MassHunter Easy Access, operated by a bioanalyst inexperienced in mass spectrometry, to determine the purity of the protein product. The LC/MS method was developed and optimized, using Easy Access Software for data analysis.

Figure 2 shows the Method tab of the Administrator window of Easy Access software. The optimized method parameters entered on this tab are shown in Table 1. Each batch was analyzed with an Agilent 1290 Infinity LC coupled to an Agilent 6530 Accurate-Mass Q-TOF LC/MS, using these instrument conditions. Once the method is optimized and saved, the following sequence starts as shown in Figure 1. In the present study, three different lots (preclinical, non-GMP, and GMP) of a model therapeutic protein were analyzed for purity.

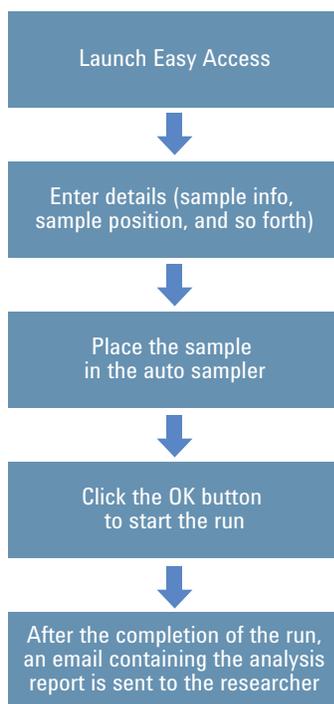


Figure 1. Easy Access workflow for lot-to-lot protein analysis.

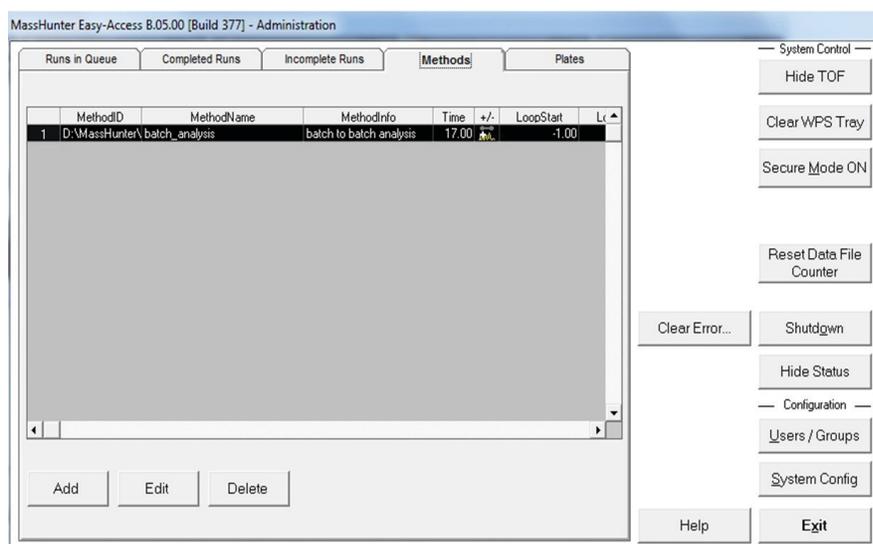


Figure 2. MassHunter Easy Access administrator window, showing the method tab.

After the samples were placed in the autosampler, the analyst entered sample details, sample position, notebook number, and analysis method for the protein studied in the Easy Access window (Figure 3), and initiated the analysis. The system was configured to send emails to the user with the report and data, once the analysis was complete.

Figure 4 shows an example report, customized for the results of one sample analysis and its match sequence, which identified the protein for a preclinical sample. The customized report contains data file name, sample name, position of the sample, and the methods used for acquisition and data analysis. Reports also include a table listing the matched protein sequence as well as a compound table listing the different molecular species of protein (intact or adduct formed species) and their relative abundance. Reports may also include the total ion chromatogram (TIC) and total compound chromatogram (TCC) for compounds identified. Finally, the report shown in Figure 4 also includes the impurities that were not matched with the protein for its intact mass or any known protein modification, suggesting some process-related impurities in the sample.

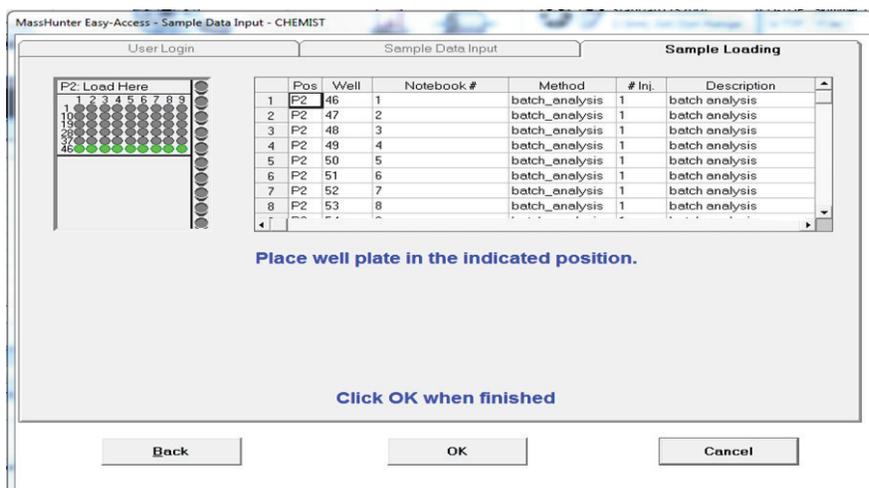


Figure 3. MassHunter Easy Access user window, showing the sample position, notebook number, and method.

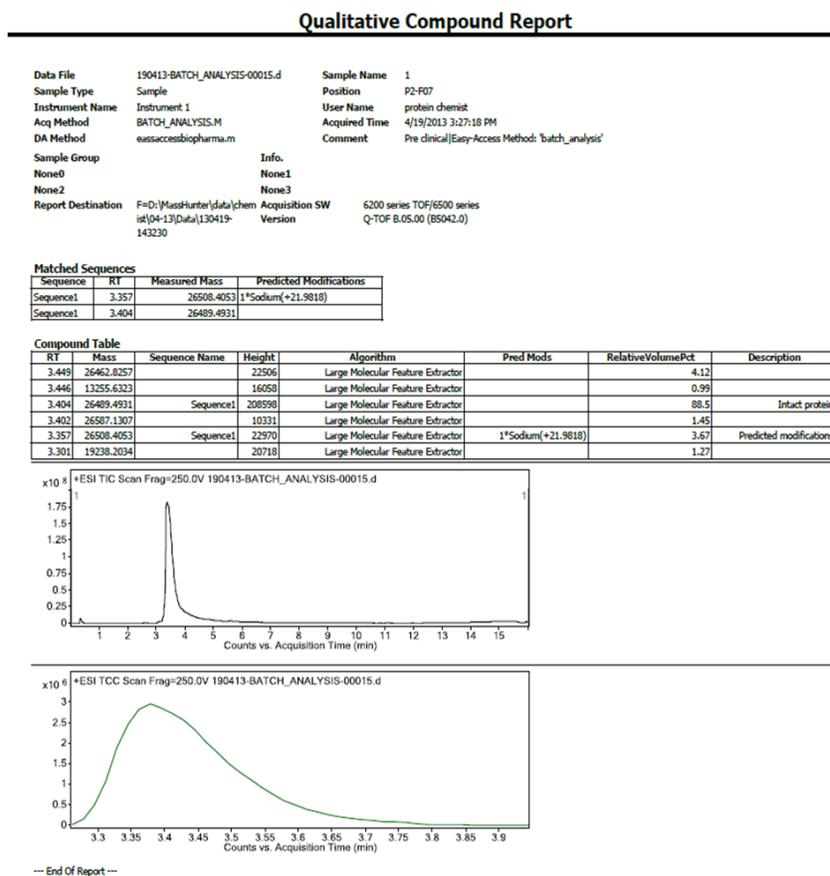


Figure 4. Customized report, showing the sample info, sample position, and method for entities that predicted modified/intact protein and impurities.

Reports such as these help the researcher to optimize purification and other process methods, helping to identify the process adjustments necessary to remove product impurities in future batches. As illustrated by the results in Table 2, the non-GMP and GMP lots analyzed after the preclinical samples showed 100 % purity.

Conclusions

This Application Note demonstrates the use of Agilent MassHunter Easy Access Software for showing consistency in lot-to-lot purification methods. Easy Access software has been proven to provide a convenient way to analyze LC/MS data for analysts who are inexperienced in LC/MS technology. It minimizes analytical run times, and improves lab productivity. As the data in this analysis show, the Agilent 1290 Infinity LC coupled to an Agilent 6530 Accurate-Mass Q-TOF LC/MS provides excellent separation and mass accuracy. Such data supports lot-to-lot consistency, and helps to determine necessary process adjustments as a result. Finally the Agilent MassHunter BioConfirm software provides automated data extraction, deconvolution and protein confirmation.

Table 2. Protein purity of the lots analyzed

Lot name	Purity* (%)
Preclinical	92
Non GMP	100
GMP	100

*Protein purity includes all the molecular species of protein (intact and its adducted species) as given in the analysis report

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