Universal data-independent LC-MS/MS workflow for host cell protein characterization and quantification in biopharmaceutical product purification process

SCIEX

Lei Xiong[1]; Yihan Li[1]; Stephen Tate[2]; Todd Stawicki[3]; Edita Botonjic-Sehic[4]; Mark Schofield[4]; Vincent Ravault[5]; Sean McCarthy[3]; Hua-Fen Liu[1]
[1]SCIEX, Redwood City, CA; [2]SCIEX, Concord, ON; [3]SCIEX, Framingham, MA; [4]Pall Corporation, Westborough, MA; [5]Pall Corporation, Cergy, France

ABSTRACT

Host cell proteins (HCPs) are a category of process related impurities that are present in biopharmaceutical products, with uncertain impacts to product quality and patient immunogenicity. Regulating agencies require drug companies monitor levels of HCPs through the purification of a biologic to ensure HCPs are below an acceptable level in the final product. Herein, we introduce a data-independent LC-MS/MS workflow for in-depth HCP characterization and quantitation in biopharmaceutical product purification process. A filter aided sample preparation (FASP) procedure is applied for sample preparation. And a one-dimensional reverse phase HPLC method with regular flow rate is used for peptide separation. With the combination of information dependent acquisition (IDA) and SWATH® based data independent acquisition (DIA), we were able to complete HCP profile characterization and quantitation up to single ppm levels.

INTRODUCTION

Host cell proteins (HCPs) are a category of process related impurities that are present in biopharmaceutical products. HCPs can co-purify with therapeutic protein of interest. And low levels of HCPs may remain during purification steps, inducing uncertain impacts to product quality and patient immunogenicity. Process changes affect HCP patterns and abundances. Therefore, regulating agencies require drug companies monitor levels of HCPs through the purification of a biologic to ensure HCPs are below an acceptable level in the final product.

Total HCP is traditionally measured by ELISA, which depends upon polyclonal antibodies. However, HCP ELISA can be dominated by highly abundant and highly immunogenic proteins, cannot provide HCP profile characterization or supply quantification of individual HCPs.

Herein, we introduce a data-independent LC-MS/MS workflow combining SWATH acquisition and 1D regular flow HPLC for in-depth HCP profile characterization and quantitation up to single ppm levels.

MATERIALS AND METHODS

Sample Preparation:
CHO S cell culture supernatants and downstream samples were desalted and digested following a filter aided sample preparation (FASP) procedure (Figure 1). After loaded on to the membrane of the molecular weight cutoff filter, the HCP samples were processed through buffer exchange, protein denaturation, reduction and alkylation, digested by trypsin/Lys-C. After high speed centrifugation, the digested samples are collected and subjected to LC-MS/MS analysis.

HPLC Conditions:
A one dimensional regular flow HPLC system coupled with TripleTOF® mass spectrometer was used for data generation (Figure 2. A). A three-hour length double gradient (Figure 2. B) was applied for protein separation and column clean up.
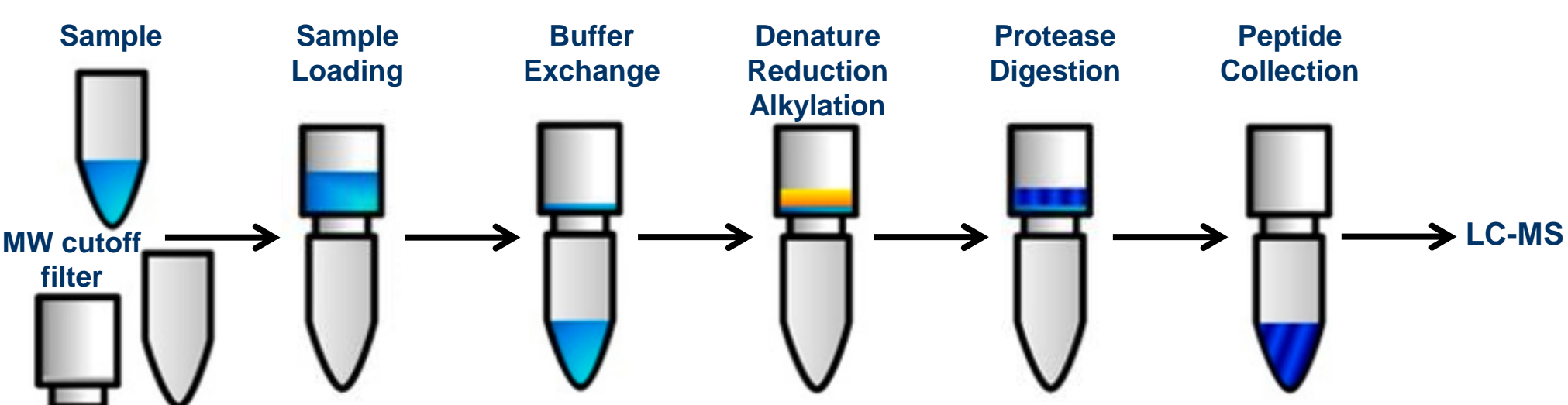

Figure 1. Filter aided sample preparation (FASP) workflow for HCP desalting and digestion.
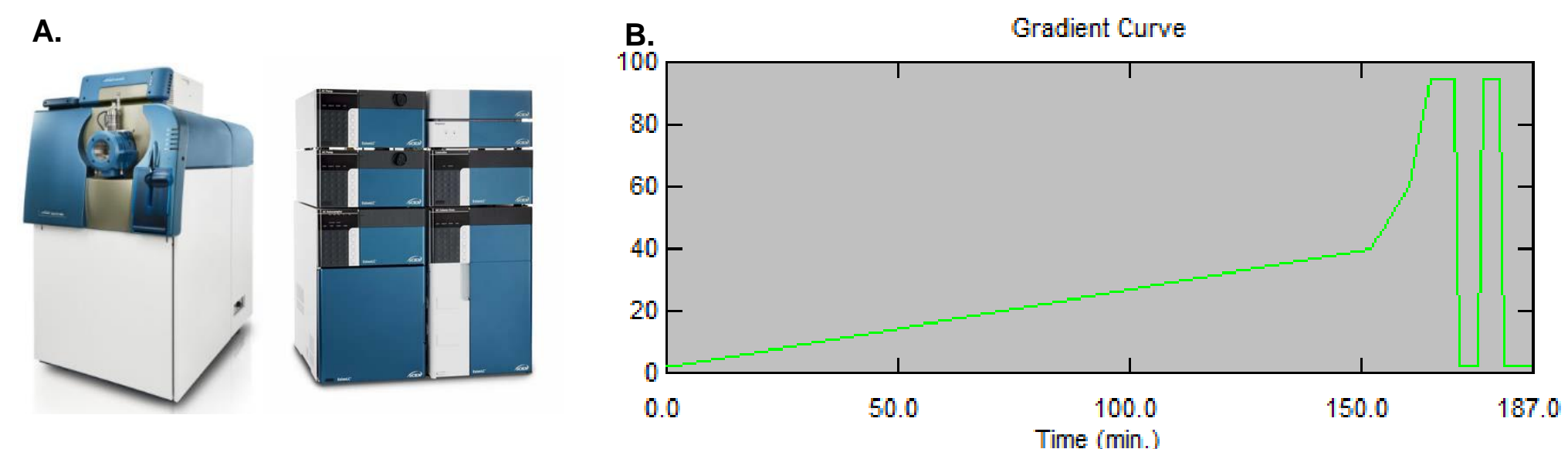

Figure 2. A. LC-MS hardware platform for HCP workflow. **B.** Three hours length HPLC double gradient: first linear part is for peptide separation, the second part is for column cleanup.

MS/MS Conditions:
An information dependent acquisition (IDA) method was used for HCP identification/characterization. SWATH® data-independent acquisitions (DIA) were subsequently performed to generate quantitative data. The method parameters are shown in Figure 3. The IDA data were interrogated against a protein database to create an ion library. The SWATH data were processed by PeakView® and MultiQuant™ software to quantify target HCP levels.
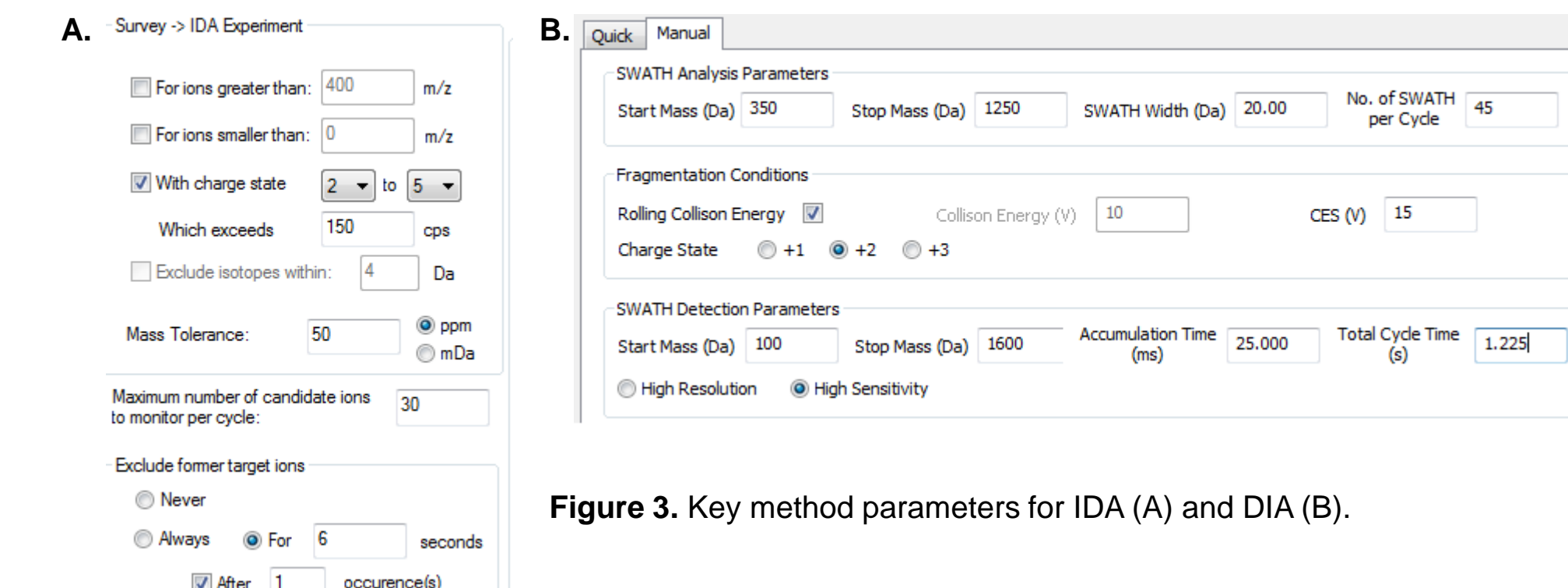

Figure 3. Key method parameters for IDA (A) and DIA (B).

RESULTS

Experimental setup optimization
During FASP sample preparation, the experimental reagent/supply and each individual step in the FASP procedure was well evaluated to achieve optimal digestion efficiency and desired sample cleanliness. Samples with a wide range of protein complexity levels were digested prior to direct injection on the LC-MS/MS system. A one dimensional regular flow HPLC was coupled with a TripleTOF® 6600 system, making the workflow straight forward to set up. Moreover, no protein fractionation is needed prior to the LC-MS/MS analysis. The protein database search result show the identification of over 1000 HCPs in the feedstock sample, and the SWATH data provide quantitation of HCPs at single ppm level.

HCP quantitation strategy and data processing workflow
Unlike the targeted protein quantitation workflow which requires spiking of specific target protein standards, the workflow presented here can serve as a generic setup to estimate protein level of all identified HCPs (Figure 4). A BSA protein standard was spiked into each sample at a known ppm level prior to protein digestion, serving as single point calibrant. The response for each protein was represented by summing the 3 most dominant fragment ions from its 3 most widely identified peptides. The concentration of each protein was estimated by comparing its response against the response of BSA. At the same time, BSA peptides were served as retention time calibrants to accommodate any retention time discrepancies of HCPs among SWATH and IDA runs.

The HCP ion library was generated by utilizing the ProteinPilot™ database search engine, and was applied to PeakView® software for SWATH data processing (Figure 5). The XICs were automatically generated and the peak areas of abundant fragment ions from signature peptides were exported for downstream statistical analysis and quantitation. MultiQuant™ software was used for accurate quantification into peak area of signature fragment ion can de automated extracted and calculated by the software (Figure 6). Changing trends of protein levels were clearly demonstrated among the HCP purification stages.
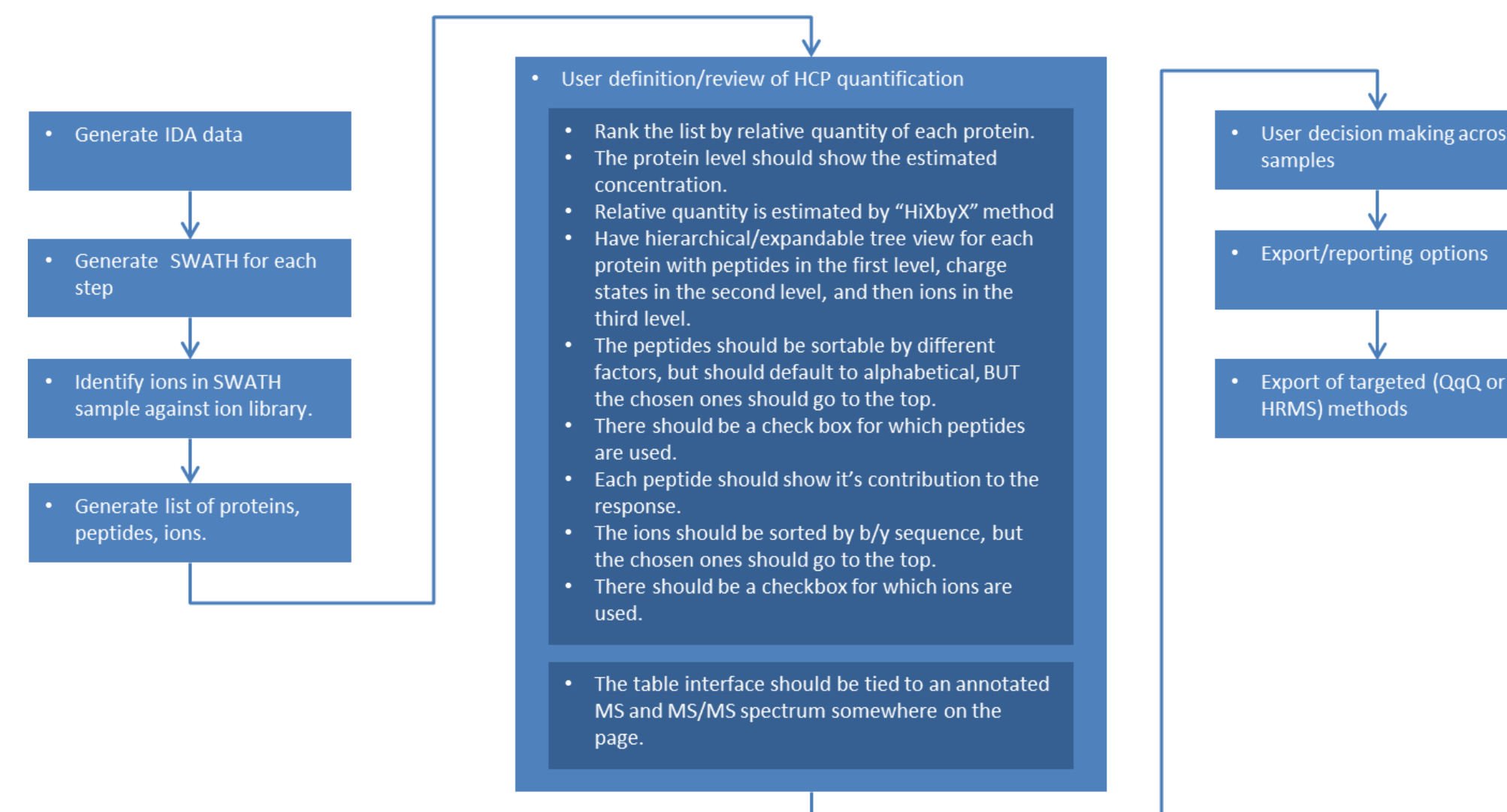

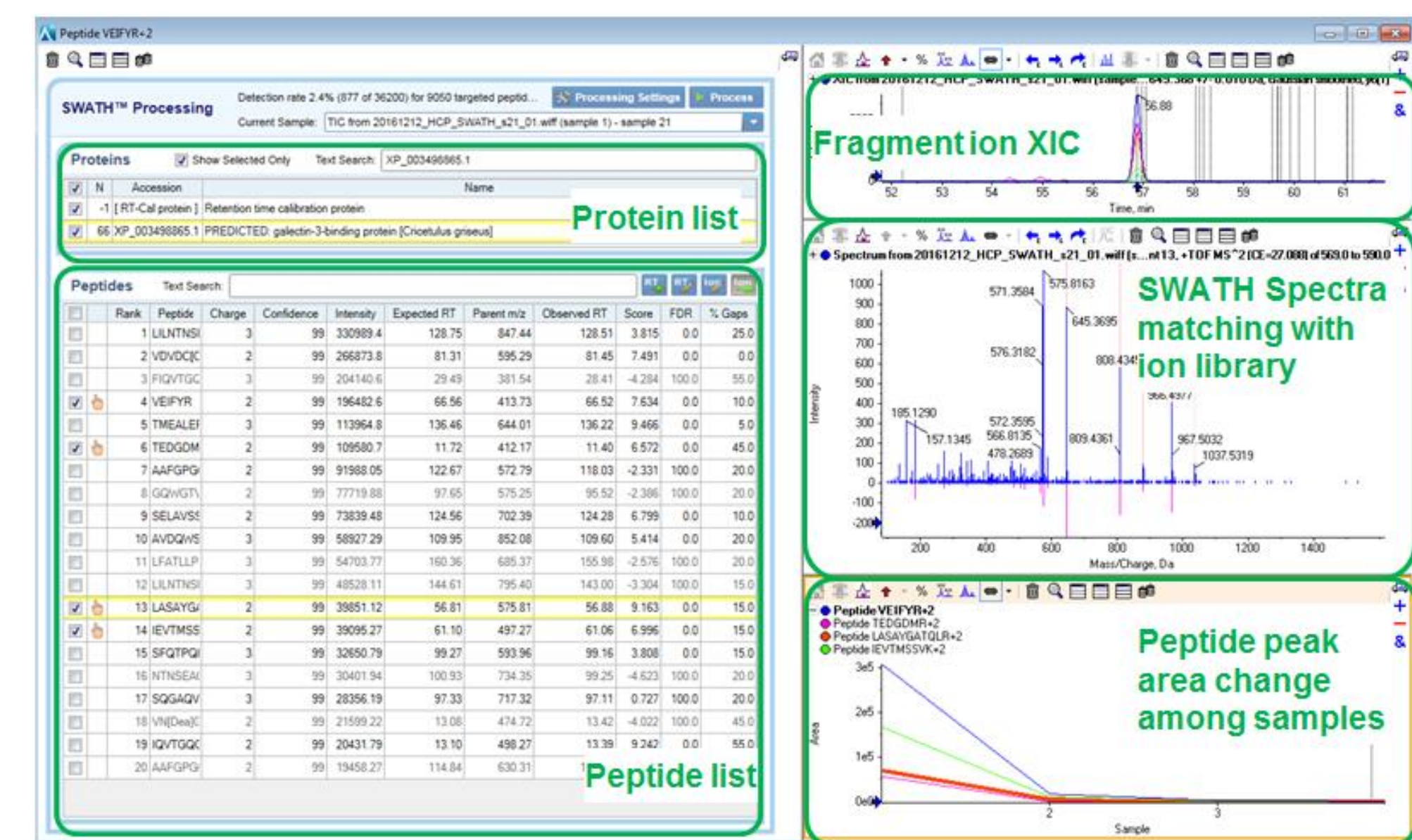**Figure 4.** Example HCP sample analysis and data processing workflow.

Figure 5. Example HCP data processing workflow by using SWATH mini apps in PeakView® software.
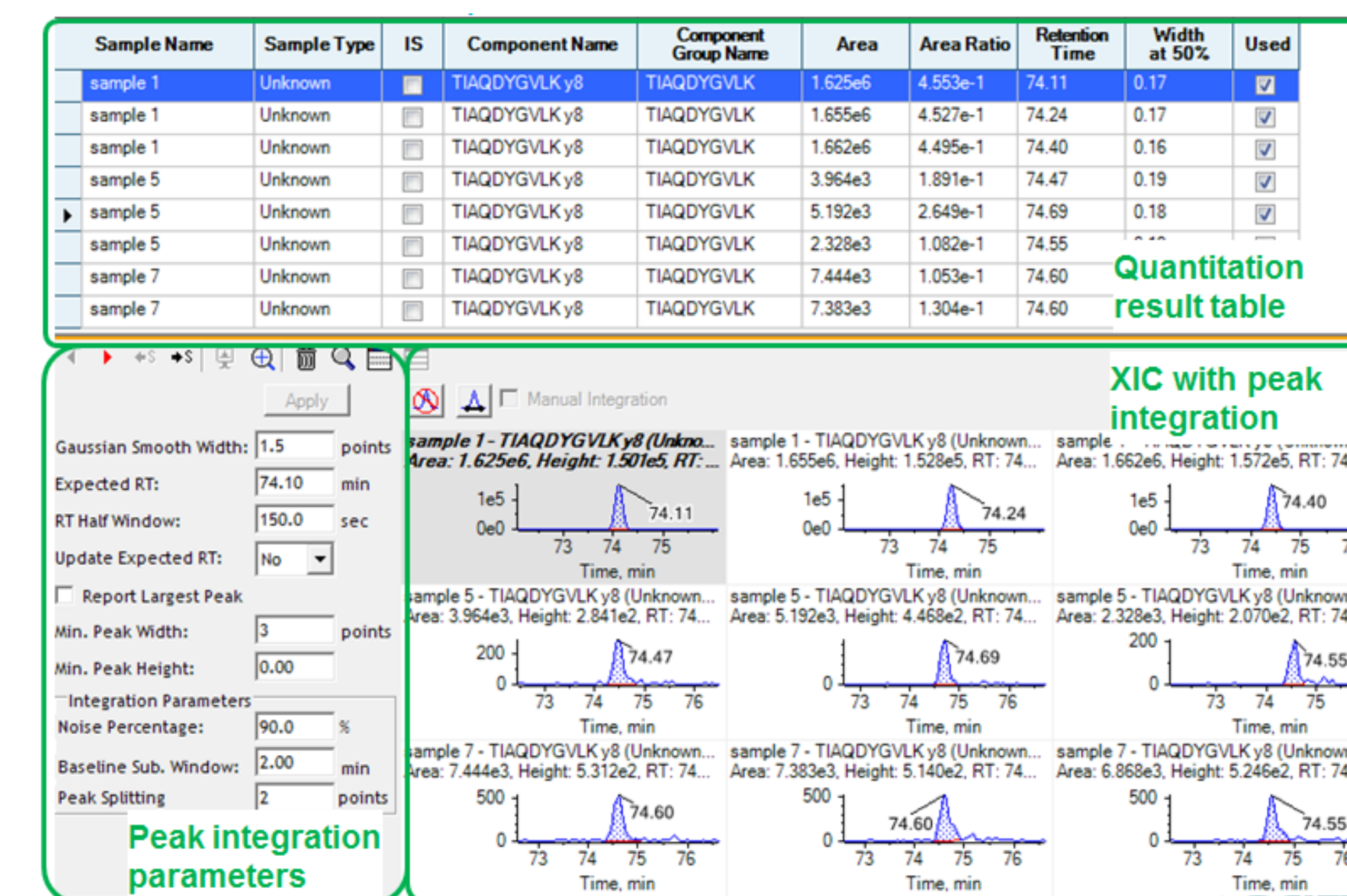

Figure 6. Example HCP quantitation workflow by using MultiQuant™ software.

CONCLUSIONS

A sensitive, simple, universal workflow for host cell protein characterization and quantitation in real biopharmaceutical product purification process is presented. A filter aided sample preparation (FASP) procedure is applied for sample preparation. And a one-dimensional reverse phase HPLC method with regular flow rate is used for peptide separation. With the combination of information dependent acquisition (IDA) and SWATH® based data independent acquisition (DIA), HCP profile characterization and quantitation in biopharmaceutical product purification process can be achieved .

TRADEMARKS/LICENSING