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Host Cell Protein Identification in Biopharmaceuticals Using Mass Spectrometry: An Iterative Acquisition Approach for Comprehensive Profiling

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Introduction

Host Cell Proteins (HCPs) are critical impurities in biopharmaceuticals that can jeopardize the safety and efficacy of biologic drugs. Their accurate identification and quantification are essential to ensuring the purity of therapeutic products. This study introduces a Mass Spectrometry (MS)-based method for the comprehensive identification and characterization of HCPs in biologic drug production. By employing an innovative iterative acquisition strategy, this approach utilizes high-resolution, high-accuracy tandem MS to detect a wide array of HCPs, including those present in low abundance. The method streamlines the workflow, making it suitable for both novice and expert users, with wide applicability in industrial settings. Ultimately, this strategy provides deeper insights into HCP profiling, supporting process optimization and compliance with regulatory standards.

Objective

The primary objective of this study is to develop a mass spectrometry-based iterative acquisition workflow tailored for the in-depth identification of host cell proteins (HCPs) in biopharmaceutical processes. This method is designed to improve detection sensitivity and achieve deeper coverage, particularly for low-abundance HCPs that are often missed in conventional approaches. By capturing a broader spectrum of impurities across multiple purification stages, the workflow enhances overall process understanding.

Experimental

Sample Collection: Monoclonal antibody (mAb) samples were obtained from the Protein A affinity eluate (Stage 1 chromatography) and the final purified material from Stage 3 chromatography.

Sample Preparation: Samples were reduced with DTT, alkylated with iodoacetamide (IAA), and digested with trypsin to generate peptides suitable for LC-MS/MS analysis.

Experimental

Mass Spectrometry: Peptides were separated on an AdvanceBio Peptide column (2.1 × 150 mm) over a 65-minute gradient and analyzed using the Agilent 6545XT AdvanceBio LC/Q-TOF system. Data-dependent acquisition was configured with 15 precursors per cycle with 1000-count intensity threshold, dynamic exclusion after one spectrum, and precursor release after 0.11 minutes. Both iterative acquisition (3 runs) and Auto MS/MS (in triplicate) were performed using the same settings for each sample.

Iterative acquisition: Iterative acquisition is a data-dependent LC-MS/MS approach where the instrument's onboard computer directs multiple runs, excluding previously identified precursor ions from MS/MS to enhance peptide and protein coverage

Data Analysis: Acquired data were searched against the Cricetulus griseus protein database using PEAKS Studio 10.2

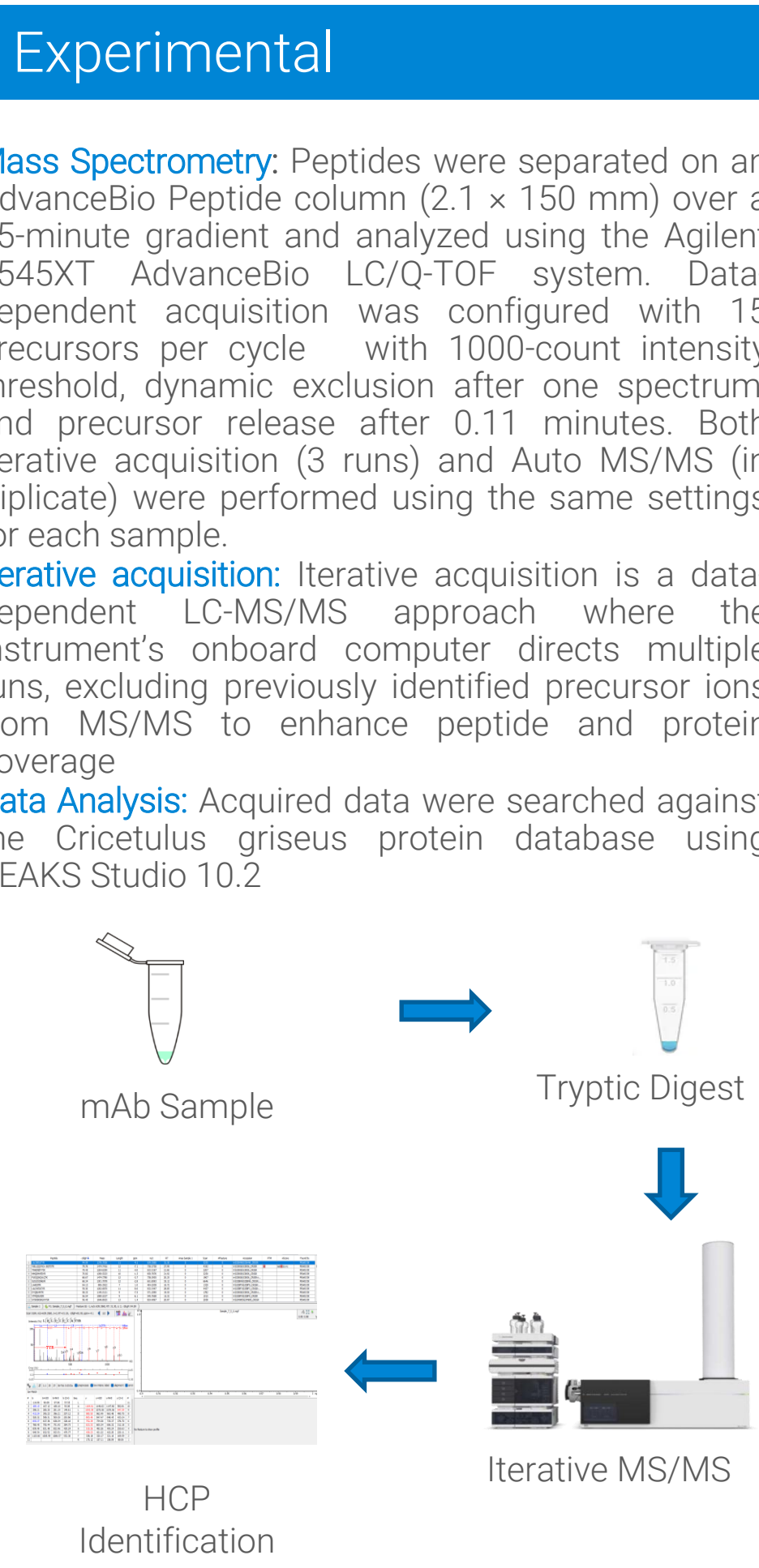

Figure 1: Schematic Representation of the HCP Analysis Pipeline

Results and Discussion

The iterative acquisition strategy demonstrated a clear advantage over the Auto MS/MS approach by identifying a significantly higher number of host cell proteins (HCPs) across both purification stages (Table1). In addition to increasing the number of identifications, it also resulted in markedly improved protein sequence coverage (Table2).

Results and Discussion

This enhanced performance is attributed to the exclusion of high-abundance precursors that were already fragmented in previous runs, thereby enabling selective targeting of low-abundance precursors in subsequent acquisitions, illustrated in Figure2. The TIC of MS/MS from three consecutive iterative runs further highlight this shift in precursor selection. Importantly, several HCPs that remained undetected in the Auto MS/MS method were confidently identified in the iterative workflow with at least two unique peptides Table2.

Stage 1 Purified Product	
Acquisition Strategy	Number of Identified HCP
Auto MS/MS (DDA)	76
iterative Acquisition	306

Stage 3 Purified Product	
Acquisition Strategy	Number of Identified HCP
Auto MS/MS (DDA)	45
iterative Acquisition	156

TABLE 1 : Number of HCPs identified with ≥2 unique peptides using Auto MS/MS versus iterative acquisition during Stage 1 and Stage 3 purification steps.

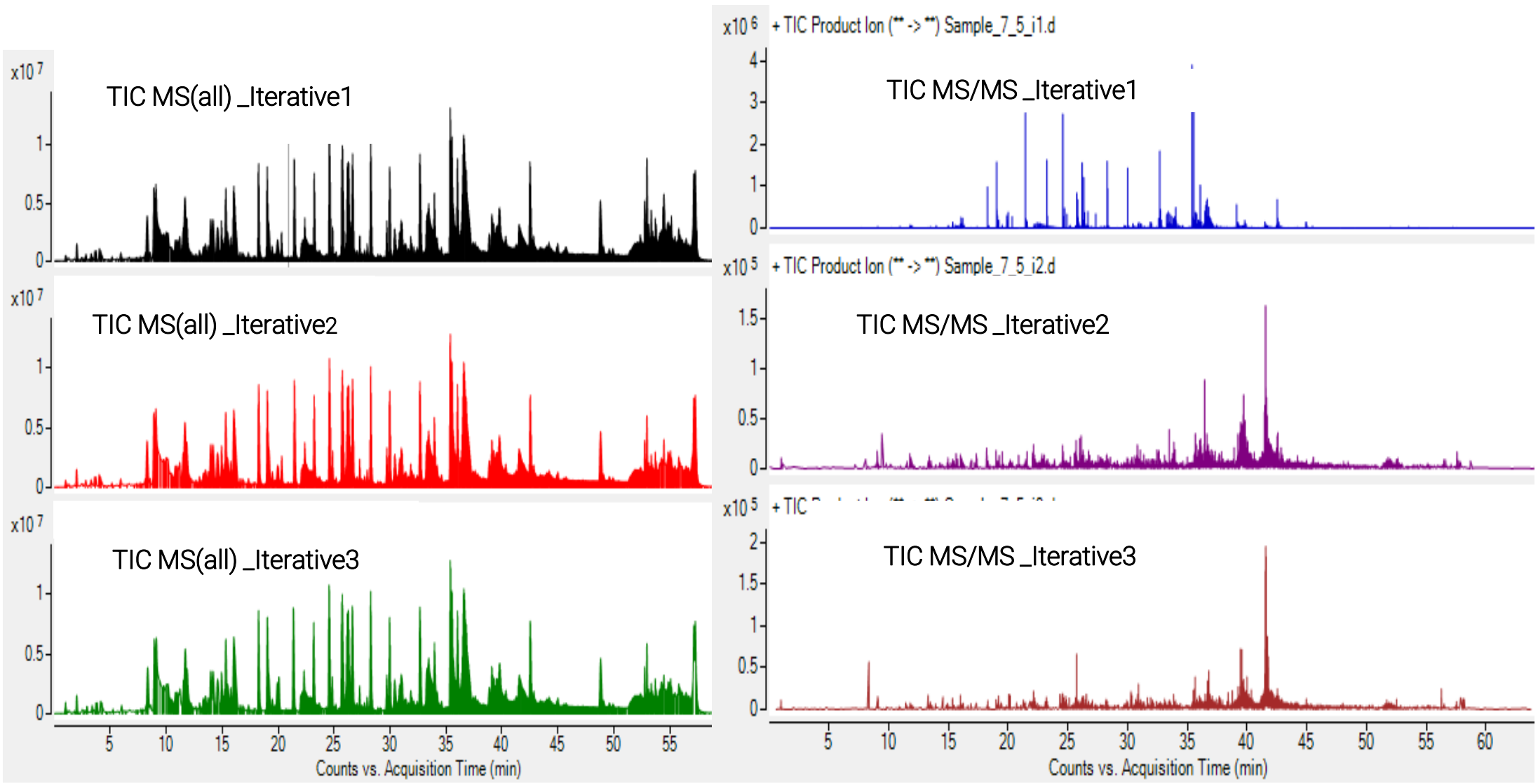

FIGURE 2: ITERATIVE ACQUISITION STRATEGY—(1) Total Ion Chromatogram (TIC) at the MS1 level and (2) corresponding Total Ion Chromatogram at the MS/MS level from three consecutive iterative runs.

Novel Aspects

This study introduces a high flow UHPLC and iterative MS/MS acquisition workflow that dramatically improves the detection of HCPs, especially low-abundance species often missed in conventional DDA methods. By automatically excluding previously fragmented precursors and enriching low-intensity ions in subsequent runs, this approach enhances sequence coverage and expands HCP identification across purification stages. The workflow is robust, scalable, and easily adaptable in industrial QC pipelines for deeper impurity profiling

Results and Discussion

Stage 3 Purified Product				
Protein Name [1/2/3]	Number of unique peptides identified from iterative acquisition strategy	Protein Coverage (%) from iterative acquisition strategy	Number of unique peptides identified from Auto MS/MS strategy	Protein Coverage (%) from Auto MS/MS strategy
Peripherin	3	21	NA	NA
Vimentin	3	16	NA	NA
Titin	41	2	4	<1
60S ribosomal protein L6	2	1	2	1
Receptor-type tyrosine-protein phosphatase S	2	6	NA	NA
Zinc finger protein 469	6	3	NA	NA
Microtubule-actin cross-linking	7	2	2	1
Microtubule-associated protein	2	2	NA	NA
Ras GTPase-activating-like protein IQGAP1	2	4	NA	NA
Zinc finger homeobox protein 3	2	1	NA	NA
Adenomatous polyposis coli protein 2	2	2	NA	NA
Ankycorbin	2	2	2	1
Citron Rho-interacting kinase	2	2	NA	NA
Complement C3	2	2	1	>1
Fibrillin-1	2	2	NA	NA
Serine/arginine repetitive matrix protein	6	6	2	1

Table 2 shows representative data with higher unique peptide counts and sequence coverage in the iterative method, which also enabled confident identification of several HCPs missed by Auto MS/MS.

Conclusions

The iterative acquisition workflow enables high-resolution, in-depth profiling of HCPs, outperforming traditional Auto MS/MS in both identification count and sequence coverage. This method strengthens process understanding, supports regulatory compliance, and offers a scalable solution for ensuring product purity in biopharmaceutical manufacturing.

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