



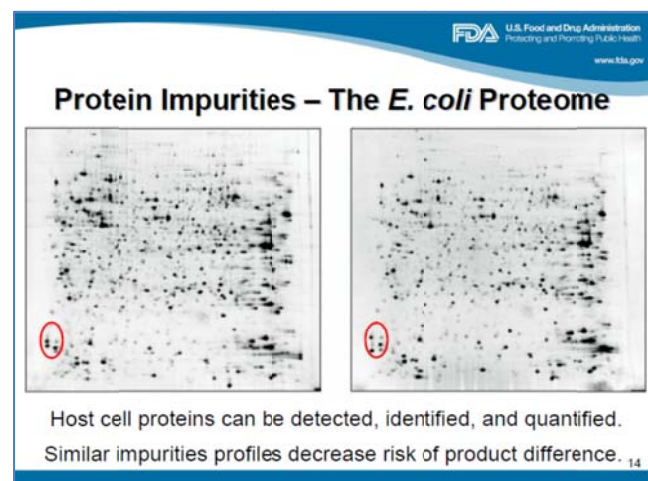
## Developing a Routine LC/MS/MS Platform for Quantitative Identification of Host Cell Proteins (HCPs)

### Introduction - Unknown HCPs are a Threat to Income

Regulators classify residual host cell proteins (HCPs) from recombinant production as process-related impurities. It is known that HCPs may elicit an inappropriate response in patients and must be monitored as part of guidelines. EMA (European Union) and FDA guidelines have been in effect since the 1990s and reflect ICH documents that the removal of HCPs should be proven:

"6.2 Validation of the purification procedure - .... The ability of the purification process to remove other specific contaminants such as host-cell proteins ... should also be demonstrated"

The repercussions of unwanted HCPs can be extremely costly, such as needing to change formulations, or repeating clinical trials. In 2006-2008 a product about to launch was the subject of scrutiny when 100% of the patients enrolled in two clinical studies developed anti-HCP antibodies [ref 2] forcing the organization to repeat trials and incur unexpected costs and delays in getting to market. The cause was excess host cell protein levels, later resolved by process changes in purification [ref 1]. When the therapeutic was marketed in the US, the USFDA was forced to respond to objections from other innovator companies, who highlighted the potential immunogenicity identified with the early version of the product [ref 3], potentially forcing a delay in launch in a new geography.



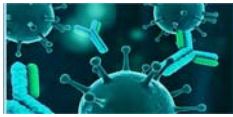
**Figure 1: Slide from an FDA Product Advisory Committee meeting in August 2012 indicating how HCP profiling can demonstrate process control .**  
[<http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/advisorycommitteeofpharmaceuticalscienceandclinicalpharmacology/ucm315764.pdf>]



Even worse, unwanted, undetected contaminants may lead to severe loss of revenue, as a biotech in the US discovered when it had to halt production of what was then the only product of its kind on the market [ref 4]. Because of supply shortages, patient groups brought litigation against the company [ref 5]. Additionally, other companies were able to leverage US legislation which allows early market entry, partly to try and prevent shortages, potentially opening markets to competitors prior to patent expiry and threatening income from what had been a unique product [Ref 6, 7, 9]. Therefore the appropriate identification and monitoring of contaminants has the potential to save significant amounts of time and money for an organization marketing a biotherapeutic.

Today many analytical methods are used for HCP analysis, such as ELISA, gels, Western Blots, and HPLC with optical detection. Some can be expensive to develop: ELISAs, for example, may take up to eight months and require the ethical housing of test animals. Additionally, antibodies raised in animals such as rabbits may not adequately reflect the situation in humans, leaving open the question of whether such animal models are appropriate. Techniques such as gels and blots may be subjective, or may require pre-knowledge about the contaminant proteins. Many of these techniques have been established as the biotechnology industry developed, reflecting the analytical techniques of their time. Some years ago, forward thinking scientists were aware of the possibilities of the use of AB SCIEX QTofs for HCP analysis. In 2008 the Laboratory of the Government Chemist (LGC) in the UK applied SCIEX iTRAQ™ techniques for the quantification of host cell protein impurities for Somatropin Growth hormone [Ref 8]. With next generation instruments, and the use of SWATH™ acquisition , AB SCIEX continues to advance this field.





## Analytical Challenges for HCP Analysis

The following challenges are associated with the analysis of HCPs:

- **COMPLEXITY:** the HCP component of a biotherapeutic is extremely complex comprising potentially of any protein or modified form, which could be expressed by the host organism. The HCP composition may change with different production and purification procedures. Therefore an analytical technique must not make any assumptions and needs to cope with the associated complexity

- **DYNAMIC RANGE:** most HCPs are present at extremely low levels. It has been suggested that monitoring should occur at least in the ppm range (1-100ppm). Therefore an appropriate analytical technique requires good dynamic range that can also cope with a dominant proportion of the therapeutic protein.

- **IDENTIFICATION:** Early techniques relied on prior knowledge of the contaminant proteins (e.g. ELISA), but an unbiased analytical technique has the potential to discover proteins that may not have been targeted, such as viral contaminants.

- **TIME/ COST:** Method development can be expensive and time consuming - therefore a generic methodology that can be applied at all stages can significantly reduce the cost, time and expertise required to profiling the HCPs.



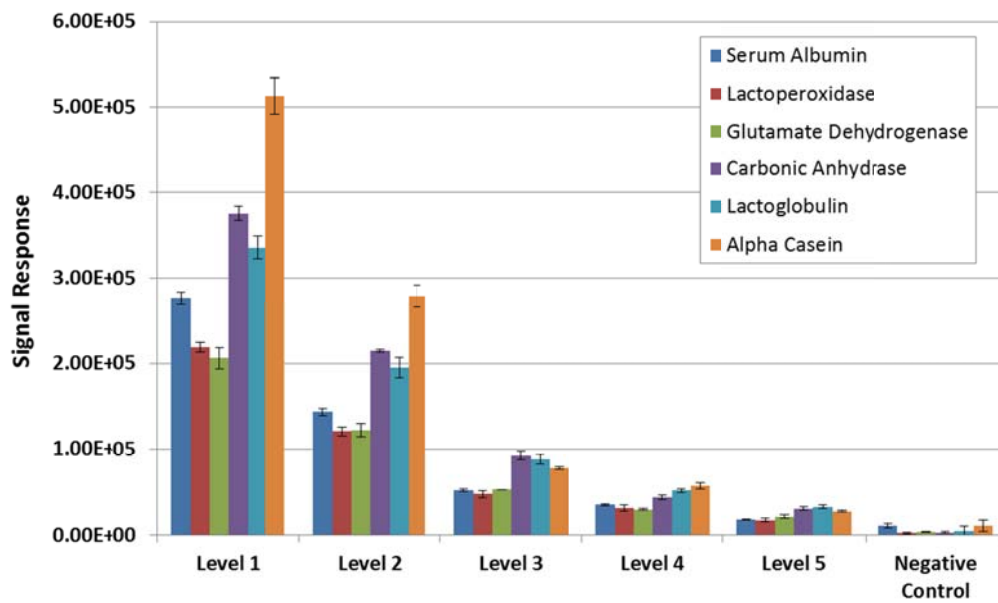
Earlier LCMS work focused on complex strategies, or on workflows developed for proteomics, some of which include two-dimensional LC. While these procedures allowed many proteins to be found they can take weeks to complete and may require careful oversight of the systems by expert users. In this technical note we demonstrate an analysis that can be used for default techniques such as peptide mapping and intact mass analysis, as well as the analysis of HCPs. The timescale of this



analysis means that the system can be used when needed, and does not need weeks of dedication to the task. Simplification of the workflow results in a robust system, minimizing the need for troubleshooting and reducing the need for expert supervision.

### Results

In this analysis we use a TripleTOF® 5600 with an Eksigent 425 LC to perform a peptide map on a series of samples that demonstrated increasing depth of coverage. To mimic a real situation of final product HCP levels, six unrelated proteins were spiked into a sample of a purified IgG of high concentration (10ug).



**Figure 1a: Dilution series of contaminant proteins showing wide dynamic range in a complex mixture with product. The error bars indicate the excellent reproducibility across 5 levels of dilution**

PPM levels of model proteins in 10ug of antibody (30' LC runs at 20ul/min):

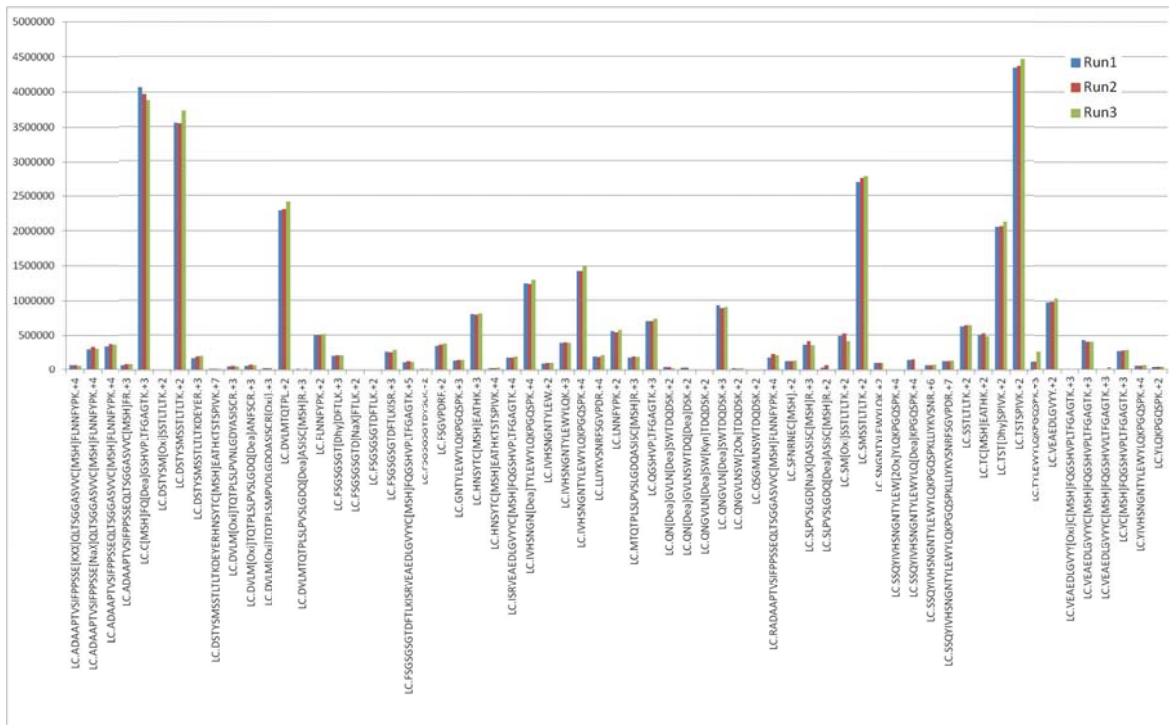
	Serum Albumin	Lactoperoxidase	Carbonic Anhydrase	Glutamate Dehydrogenase	Alpha Casein	Lactoglobulin
Level 1	415	485	182	350	148	114
Level 2	207	242	91	175	74	57
Level 3	104	121	46	88	37	29
Level 4	52	61	23	44	18	14



Level 5	26	30	11	22	9	7
---------	----	----	----	----	---	---

**Table 1: Dilution series of contaminant proteins showing wide dynamic range in a complex mixture with product.**

To prove that the analysis is both quantitative, and appropriate for unambiguous identification of proteins, the spiked in proteins were analyzed at a series of points reflecting a wide range of concentrations in a realistic study (table 1). Figure 1 shows how the identification of proteins is robust and reproducible despite the decreasing concentration of proteins unrelated to the biotherapeutic. It must be noted that this was achieved without complex chromatographic means, and without interfering with the peptide mapping of the biotherapeutic protein itself. In addition, when using the amount of biotherapeutic as a measure against which to normalize, the identified contaminants were consistent, and the coefficients of variation in the repeat analyses was consistently low. This is reflected in Figure 2 where a wide variety of peptides with and without modifications can simultaneously be identified and quantified. The efficiency gains for an organization and the enhancements to monitoring by using this approach can therefore be very large.



**Figure 2: Reproducibility and Dynamic range are demonstrated by the ability to quantify specific peptides and modified peptides as part of a routine peptide map. All CVs are below 10% in this graph over a wide range of peptide sizes and modifications.**

The use of SWATH™ provides the sequences of the peptides being analyzed and can therefore provide better proof of the identity of the proteins. SWATH has been developed to provide MSMS information throughout an analytical run and does not require pre-knowledge of the sample by the user, reducing the technical burden, as well as providing a level of automation that is welcome to any lab requiring improved throughput.

### Conclusions

By improving their analytical strategies, organizations can save significant amounts of money by monitoring Host Cell Proteins at all stages of development. The use of LCMS on an AB SCIEX QToF has long been shown as an effective technique that can identify and quantify individual proteins to provide a detailed catalogue of contaminants (ref



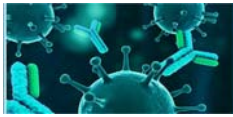
2006). In this new work, a comprehensive method of analysis has been developed that provides a straightforward platform to use. The simultaneous detection and quantification of HCPs in a sample contributes to the greater safety of the product, and has the potential to save significant time and costs in HCP analysis.

### Appendix: Instrument Conditions

- TripleTOF<sup>®</sup> 5600 System with the Turbo V<sup>™</sup> Source.
  - 65 micron Probe
- Eksigent ekspert<sup>™</sup> 425 System
  - 5-50ul Gradient Flow module in Direct Inject mode
- Column: 0.5mm X 100 mm, 3µm, 120Å ChromXP<sup>™</sup> C-18
  - **Flow: 15 µL/min** ; Column Oven at 40 °C
- Solvent A: 2%ACN 0.1% FA
- Solvent B: 98%ACN 0.1% FA
- TripleTOF Parameters:
  - One 0.16 second MS scan
  - 30 0.08 second MSMS SWATH scans, 20 m/z width
  - Total Cycle Time 2.56 seconds.

### References:

- 1) Vol. 2 November 2010 The British Journal of Clinical Pharmacy 2010 summary study – high levels of HCPs in the powder form;  
[http://www.clinicalpharmacy.org.uk/volume1\\_2/2010/november/p298-301\\_drugappraisal\\_web.pdf](http://www.clinicalpharmacy.org.uk/volume1_2/2010/november/p298-301_drugappraisal_web.pdf)
- 2) Omnitrope discussions EMA; [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Scientific\\_Discussion/human/000607/WC500043692.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000607/WC500043692.pdf)
- 3) Pfizer objection to Omnitrope in a letter to the FDA;  
<http://www.fda.gov/ohrms/dockets/dockets/04P0231/04P-0231-pdn0001.pdf>
- 4) [Xconomy. Com:](http://www.xconomy.com/boston/2009/06/16/genzyme-halts-production-at-allston-drug-plant-after-virus-appears/) Production Halt for Genzyme 2009;  
<http://www.xconomy.com/boston/2009/06/16/genzyme-halts-production-at-allston-drug-plant-after-virus-appears/>
- 5) Fierce Biotech: Genzyme/ Sanofi litigation 2012;  
<http://www.fiercepharmamanufacturing.com/story/litigation-sanofi-genzyme-negligently-allowed-virus-contamination-manufactu/2012-03-12>
- 6) <http://en.wikipedia.org/wiki/Genzyme>
- 7) Fierce Pharma – As Shire Gives up on Replagal; <http://www.fiercepharma.com/story/shire-gives-replagal-us-sanofi-stands-gain/2012-03-15>



- 8) Monitoring HCPs on a QStar instrument:  
<http://www.nmschembio.org.uk/PublicationArticle.aspx?m=288&amid=4642>
- 9) Bayh-Dole Act: [http://en.wikipedia.org/wiki/Bayh%E2%80%93Dole\\_Act](http://en.wikipedia.org/wiki/Bayh%E2%80%93Dole_Act)

For Research Use Only. Not for use in diagnostic procedures.

© 2014 AB SCIEX. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Publication number: 7460213-01