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Application note
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On-line HPLC analysis and data integration with Numera and Lucullus PIMS shown on the example of a CHO process

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Abstract

HPLC is the common reference method for product and metabolite analysis in bioprocesses. Sampling is needed for these analyses, resulting in off-line measurements along the process. In order to reduce time and cost expenses in process development and production timely availability of these HPLC data is indispensable. In this application note monitoring of a CHO process by automated sampling and sample processing with the Numera system and an on-line Thermo Scientific™ UltiMate™ 3000 HPLC system is demonstrated. High-frequency data of product and B-vitamins is shown, illustrating the benefits of automated sampling. Additionally, data integrity can be guaranteed by the Process Information Management System Lucullus (Lucullus PIMS), merging all data attached to a process including results of the on-line analytics.

Introduction

Chromatography is a tool that is commonly used in biopharmaceutical industry in the downstream processing as well as for analytics. HPLC is the prevalent reference method for quantification of metabolites, product or product quality attributes with high reliability and accuracy. Typically, HPLC analysis is performed in bundles off-line in a lab area separated from the process stream. Hence, data is available after the process run for informative reasons only. No timely intervention to the process is possible anymore.

On-line availability of HPLC data would reduce time and cost expenses for analytics and at the same time assure consistent analytical quality. If the data would additionally be available in real-time in the process management system, it could be used for in-process control e.g. for defining an abort criterion or changing feeding setpoints. More sophisticated control strategies could be implemented by receiving high-frequency analytical data.

All these requirements can be fulfilled by combining Numera, an automated sampling system that allows on-line analysis by various third-party analyzers, and Lucullus PIMS, a Process Information Management System, that merges all data in a single software. The application of both is demonstrated on the example of a CHO process for monitoring of metabolites and product via HPLC.

Hardware and Software

Numera system and UltiMate 3000 HPLC system

The automated sampling system Numera is equipped with a multiplexer, a dilution and a filtration module. An autosampler is used to collect the samples, to transfer a part of the sample to the UltiMate 3000 HPLC system (Thermo Fisher Scientific) for analysis as well as for storage of the samples at 4°C. The HPLC consists of a pump (LPG-3400SD), a column oven (TCC-3000SD) and a diode array detector (DAD 3000) (Figure 1).

The relay-based connection between Numera and the pump of the HPLC allows a direct sample injection from the Numera autosampler into the HPLC. The synchronization with an instrument method for chromatographic separation is achieved via the software Lucullus PIMS.

Chromeleon CDS and Lucullus PIMS

Apart from its features as process management system, Lucullus PIMS enables the communication between Numera and the HPLC system (Figure 2). The HPLC is controlled by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). Chromeleon CDS contains the instrument method defining the separation parameters (e.g. flow rate, temperature, gradient etc.) as well as the processing method that is necessary for peak integration and concentration calculations.

A sampling trigger is set by Lucullus PIMS, which can additionally order an HPLC analysis of the sample. The injection can be assigned as calibration standard, check standard or sample. Additionally, the dilution made in the dilution module can be defined, which is important for receiving

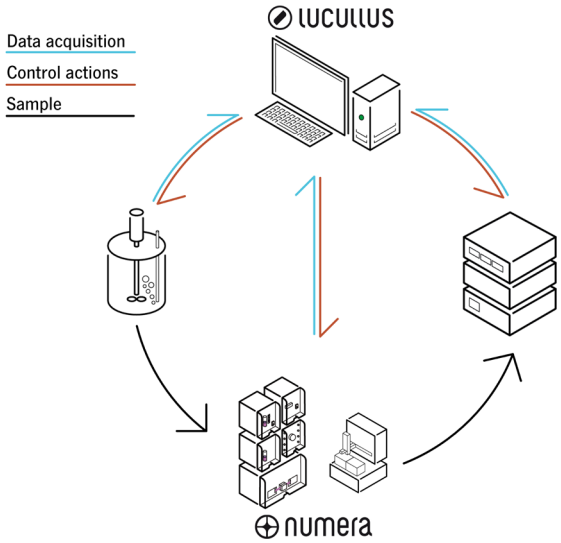

Figure 2: Overview of the interaction between the bioreactor, Numera, the HPLC and Lucullus PIMS. The sample path is shown as black arrows: the sample is drawn from the reactor, processed by Numera and collected in the Numera sampler. From there it is transferred to the HPLC and analyzed. Lucullus PIMS is the overall software, acquiring data from the bioreactor system, Numera and the analyzer (blue arrows). In addition, it is performing all control actions (red arrows) including the sample trigger and the initialization of the HPLC analysis.

Figure 1: Numera for automated sampling and on-line HPLC analysis including a multiplexer, dilution and filtration module as well as an autosampler which is connected to the UltiMate 3000 HPLC system.

the correct concentration values from Chromeleon CDS. Lucullus PIMS automatically creates a sequence in Chromeleon CDS, including the predefined instrument and processing method, as well as sample specification (i.e. calibration standard, check standard, unknown) and dilution. The sample name resembles the automatically assigned barcode of the sample in Lucullus PIMS.

Once the HPLC analysis is completed, the system can be kept "ready", in "smart standby" or it can be shut down ("smart shutdown") until the next sample is sent. The concentration value that was calculated through the processing method is sent back to Lucullus PIMS. Hence, it is accessible to the user directly in the process management system and can be used further if necessary (e.g. control strategies).

Materials and Methods

Set-up

Cultivations were performed in a 3.6 L bioreactor equipped with a pH and a pO_2 probe as well as a sampling nozzle that is connected to the Numera sampling system. HPLC analytics were performed on-line with the connected UltiMate 3000 HPLC system. The reactor is supplied with air, O_2, CO_2 and N_2 to control dissolved O_2 and CO_2 at 40% and 12.5%, respectively. Acid and base, as well as three feed-back controlled feeds are connected to the bioreactor. All devices are connected to Lucullus PIMS.

In Lucullus PIMS a sampling plan is created, which defines when a sample should be drawn, which preprocessing (e.g. dilution, filtration) should be performed and which analytics should be done. Lucullus PIMS later triggers the sampling event and collect all data for further applications. This includes samples taken by Numera or manually drawn samples.

CHO process and analytics

A batch process and a fed-batch process were performed, feeding glucose, glutamine and other amino acids. During the fed-batch process a pH shift was performed after ca 200h. Automated sampling was performed every hour and manual sampling approximately every 12h. The HPLC method for IgG production was performed according to Kroll et al. [1] and the vitamin analysis as described in Hofer & Herwig [2].

Results

The narrow tubings as well as the low sample volumes of the automated sampling system leave room for a minor sample dilution (~ 5%). In order to receive the correct concentration values, the HPLC calibration can be performed via Numera (i.e. standards are processed via Numera before analysis). Due to the high precision of the system, the dilution factor can also be calculated and inserted in Lucullus PIMS to receive the correct results. Both methods result in very high accuracy of the system as shown in Figure 3 on the example of IgG. The calibration method leaves an error of 0.03% and the method calculating a dilution factor reduces the error to even 0.01%. These errors are inside the normal error range of analytical devices.

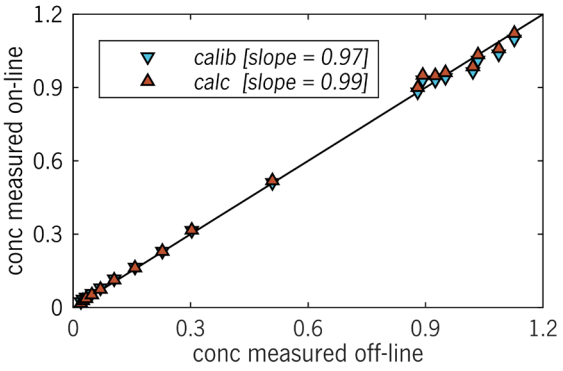

Figure 3: Comparison of the concentrations of manual samples measured off-line and automatically drawn samples measured on-line. Both analyses are in perfect accordance, if minor sample dilution via the system is considered. Compensation of the sample dilution can be performed by processing the standards for calibration with Numera resulting in an offset of 0.03% (light blue upside-down triangles) or by calculation of the dilution factor resulting in an offset of 0.01% (red triangles).

In the batch process different, B-vitamins were analyzed, namely niacinamide, folic acid, B12 and riboflavin. These substances often range in very low concentrations, meaning also little changes in concentration. In Figure 4 the differ-

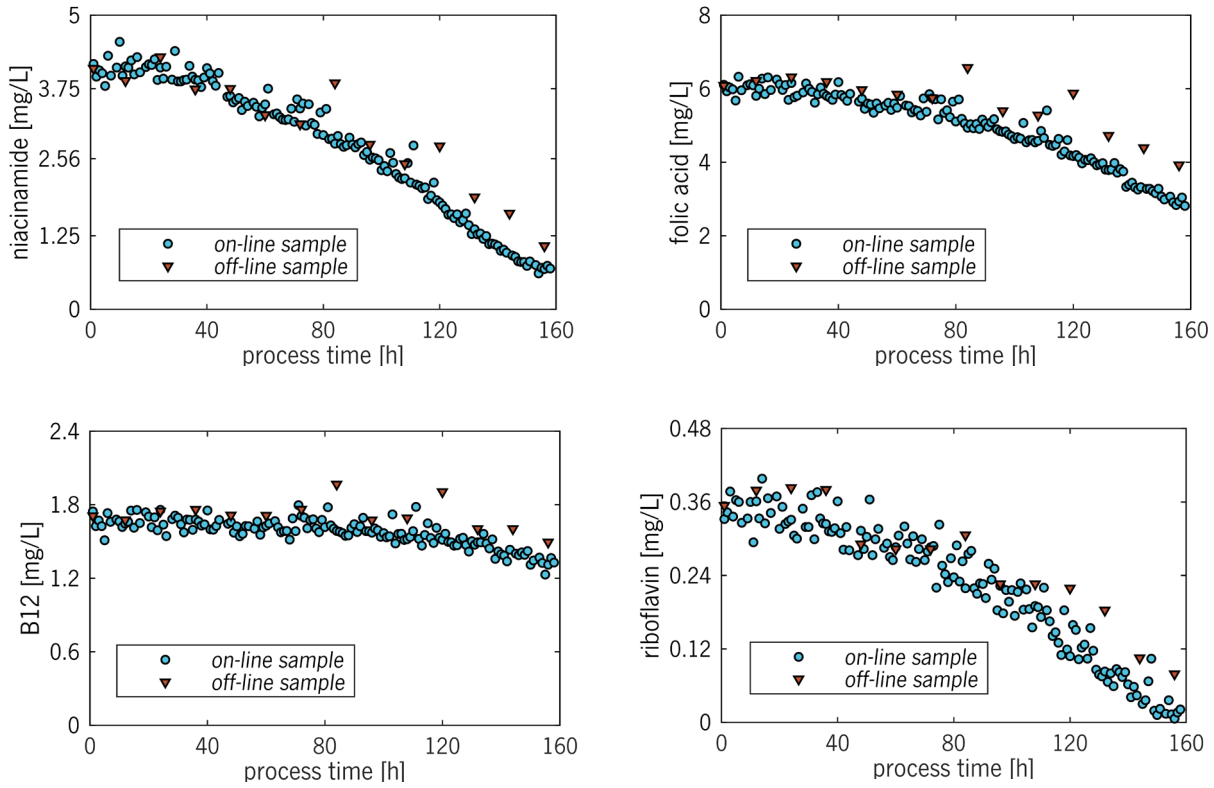

Figure 4: Different vitamins were analyzed over process time. An uptake of niacinamide, folic acid as well as riboflavin can be observed considering the high-frequency on-line samples (light blue dots). This observation is harder to follow with the manual samples (red upside-down triangles).

ences between on-line and off-line sampling can be clearly seen. The high sampling frequency as well as the reduction of operator variabilities lead to a smooth observation over time and allow conclusions about metabolic behavior of the cell (uptake or release).

In the performed fed-batch process, the automated sampling system was running idle over 330h, drawing and analyzing one sample per hour. Additionally, the system was running unattended for 50h (between 160h and 210h process time), delivering reliable analytical data.

The product IgG was analyzed on-line by Numera and the UltiMate 3000 HPLC system. Manually drawn samples were easily measured at-line, inserting the sample in the Numera autosampler and giving the inject command via Lucullus PIMS. Of course, these data were also accessible there. Figure 5 shows the IgG data over 330h of process time.

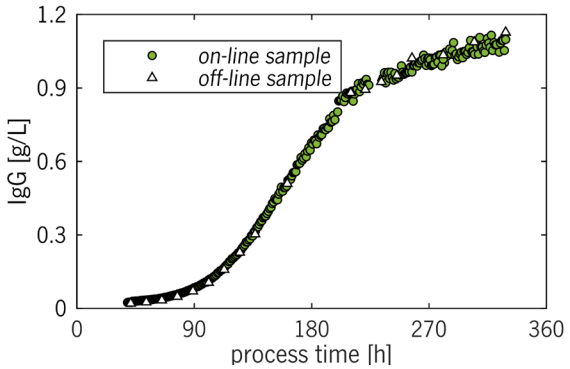

Figure 5: The production of IgG over process time can be seen with the on-line as well as the off-line data. The results are in good accordance. Between 160h and 210h the process was unattended; hence, just automated samples were drawn.

Analyte	Mammalian cells	Yeast	Microbials	Extremophiles	Fungi	...
glucose	•	••	••	•	•	
glycerin		••	•	•		
galactose	•		•	•	•	
fructose	•		•	•	•	
sucrose	•		•	•	•	
lactose			•	•	•	
xylose			•	•		
lactate	•	•	•	•		
acetate			•	•		
citric acid	•	•	•	•	•	
succinate	•	•	•	•	•	
pyruvate	•	•	•	•	•	
methanol		••				
ethanol		•	•			
aceton			•			
butanol			•	•		
glutamine	••	•	•		•	
glutamate	••	•	•		•	
asparagine	••	•	•		•	
aspartate	••	•	•		•	
tyrosin	••	•	•		•	
other amino acids	••	•	•		•	
vitamins	••	•	•		•	
proteins	•	•	•			
IgG	••					
Fab	•		•			
antibiotics					•	
...						

Table 1: On-line availabilty of HPLC analysis holds a variety of opportunities for bioprocess development. The table shows examples of analytes that can be measured by HPLC for different hosts. • HPLC analysis possible •• tested (data in-house available)

Conclusion

The automated sampling system Numera can be connected to various third-party analyzers to facilitate on-line analytics, i.e. monitoring. The sampling trigger and the analytical trigger are sent by Lucullus PIMS, which also receives the measurement results. Hence, Lucullus is a process management system delivering full data integrity.

The reliability and accuracy of Numera were demonstrated on the example of an on-line HPLC analysis in a CHO process. The data show that reference analytics can be brought directly to the process (e.g. product measurement). Additionally, metabolites for process characterization and understanding can be monitored.

In development facilities, such an approach can result in more efficient experimental designs, easier handling of parallel runs and finally a reduced time-to-market.

A list of analytical possibilities of on-line HPLC for different hosts is shown in Table 1.

Key Results

– On-line availability of reference analytics (HPLC)
– High accuracy
– Data integrity (one software merging all data)
– Higher efficiency
– Reduced time-to-market

References

[1] Kroll, P. et al (2017), *"Workflow to set up substantial target-oriented mechanistic process models in bioprocess engineering",* Process Biochemistry, 62, 24–36
[2] Hofer, A. and Herwig, C. (2017), *"Quantitative determination of nine water-soluble vitamins in the complex matrix of corn steep liquor for raw material quality assessment"*, J. Chem. Technol. Biotechnol, 92: 2106-2113

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