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Application note
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Automated bioreactor sampling for on-line analysis of amino acids using two approaches: HPLC and Cedex® Bio HT Analyzer

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Abstract

To facilitate optimal process control limiting substrates must be identified, monitored and controlled. Amino acids are often critical substances, especially in mammalian processes. Measurement and thus monitoring is tedious and rarely done. In this application note, we demonstrate how two different analyzers for amino acids, namely HPLC-FLD and Cedex® Bio HT, can be connected on-line to the reactor using the automated sampling system Numera®. On the example of a CHO process, it is shown how the on-line availability of the data may increase process understanding in a shorter time and facilitate process control.

Introduction

Amino acids (AA) play a critical role in the development of bioprocesses. A prime example are CHO cultivations, in which different amino acids may be limiting and must be fed to the process. Thus, measurement and monitoring of these amino acids is essential for successful and efficient process performance. Amino acids are mostly analyzed by chromatographic methods. There are ion chromatographic methods available using amperometric detection that require no sample preparation such as derivatization steps. But these methods do often have long run times, in the range of 1h and more. Reversed-phase HPLC methods have a shorter run time, but require a derivatization step to analyze the amino acids by fluorescence or UV absorbance. The derivatization steps are often per formed manually but can also be performed in an automated manner by in-needle or in-vial derivatization with an adequate autosampler [1]. In addition, assay-based methods are available for amino acid analysis like offered by the Cedex® Bio HT. These methods require no derivatization and have similar analysis times as a reversed-phase HPLC method. Nevertheless, such assays are just realized for a small selection of analytes, commonly only glutamine (Gln) and glutamate (Glu). The choice of the right method is strongly dependent on the requirements of the process itself. In the early development phase, HPLC methods delivering information about 19 amino acids and more are more useful for process characterization and understanding. If critical amino acids are identified, the Cedex® Bio HT might be sufficient for their monitoring and controlling.

In any case, on-line availability of the analytical data is necessary in order to reduce experimental time and facilitate process control. The Numera® system enables this on-line analysis for both HPLC and Cedex® Bio HT and, in combination with Lucullus® PIMS as the central data hub, allows for monitoring, which is demonstrated in a CHO cultivation.

Hardware and Software

Numera® system, HPLC and Cedex® Bio HT

The modular Numera® system consisted of a multiplexer, a dilution, a filtration and a routing module as well as an autosampler for sample depositing and storage. The routing module holds a lot of new advantages as it resembles the sample distributor of the system. Not only does it allow the transfer of samples to additional analyzers but it also allows individual sample processing (individual combination of dilution, filtration or neither of both) for each sample in a process. For AA analysis the Thermo Scientific™ Ultimate™ 3000 HPLC system (Thermo Fisher Scientific) was equipped with a pump (LPG-3400SD), an autosampler for fraction collection (WPS 3000FC), a thermostatted column compartment (TCC-3000SD), a diode array detector (DAD-3000) and a fluorescence detector (FLD-3400RS). The sample transfer is realized by connecting the injection valve of the Numera autosampler with the fraction collector valve of the HPLC autosampler. This way, the sample is transferred to the HPLC autosampler where the derivatization and injection can be performed. The sample transfer to the Cedex® Bio HT Analyzer is realized by a tubing connection between the routing module and a fixed rack position in the Cedex® Bio HT.

Data integration

The sample trigger as well as the command for performing an HPLC or Bio HT measurement is performed by the Process Information Management System Lucullus (Lucullus® PIMS). The analytical result is also sent back to Lucullus PIMS; hence, all process-relevant data is merged in the one software. For more details about the software connection refer to Application Note #002 and #003. A schematic overview of the whole set-up can be seen in Figure 1.

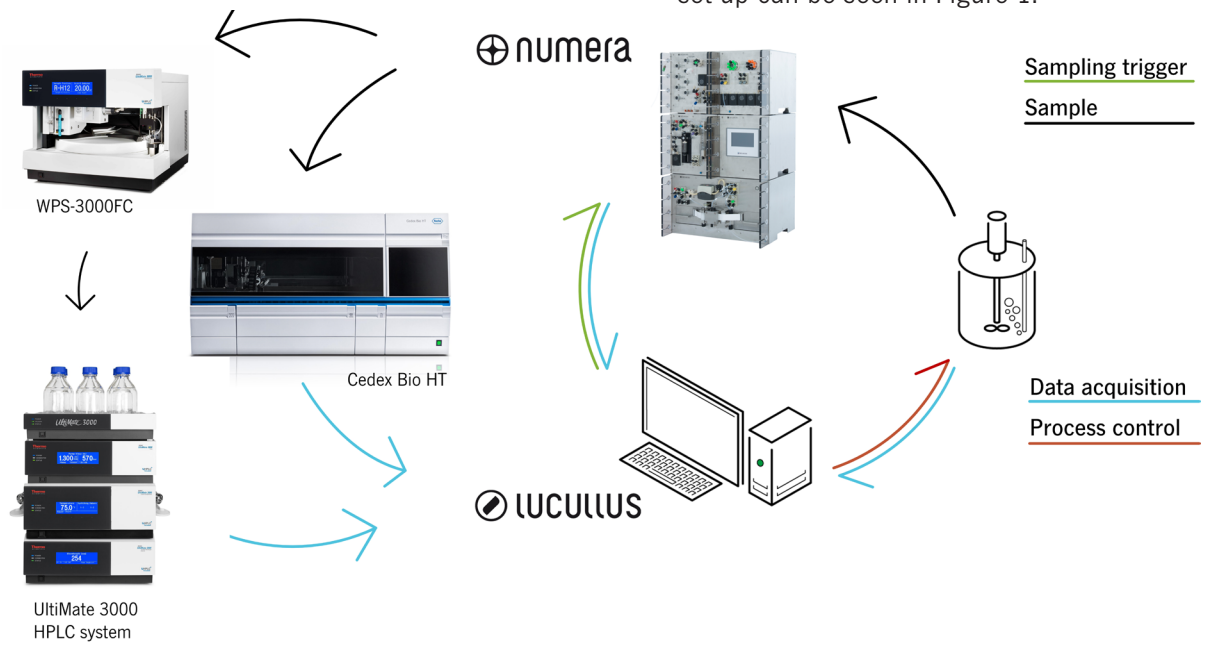

Figure 1: Lucullus® PIMS is the overall software, uniting data acquistion and control of the bioreactor system, Numera® and the two analyzers, namely UltiMate 3000 HPLC system and Cedex® Bio HT Analyzer. To implement automated analysis with both devices, Numera is equipped with a routing module, which allows multiple sample distributions, e.g. to the Bio HT. Amino acid analysis by HPLC is realized by transferring the sample from Numera to an analytical autosampler with fraction collector (Thermo Fisher Scientific), where the sample is derivatized and injected.

analyzer	analytes (AA)	Sample preparation	Time for sampling & analysis	LOQ
HPLC	Ala, Arg, Asn, Asp, α-AAA, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val	Filtration Derivatization (OPA) Diliution (via Numera or HPLC)	45 min	0.006 mM
Bio HT	Glu, Gln	Diliution (via Bio HT) Filtration is not necessary	25-30 min	0.1 mM

Table 1: Comparison of the features of HPLC and Cedex® Bio HT for on-line AA analysis. HPLC facilitates the analysis of many AA even with low concentrations. Though, sample preparation is more tedious also increasing the time for analysis. The Cedex® Bio HT can be applied for two AA so far with a higher limit of quantification. However, sample preparation is easy and partly done by the system itself (e.g. dilution). This way, time for sampling and analysis can be reduced.

Materials and Methods

CHO cultivations

CHO cultivations were performed in a 3.6 l bioreactor that is connected to the Numera system, an HPLC and the Bio HT. The system was supplied with air, O2, CO2 and N2 to control dissolved O2 and CO2 at 40% and 12.5%, respectively. The pH was controlled on 7.00 with acid and base and temperature at 37°C. All devices were connected to Lucullus® PIMS. Batches as well as fed-batches were performed, applying three feeds including glucose, glutamine and other amino acids. All feeds were controlled in a closed-loop approach. Automated sampling and analysis were applied every 2h or every 3h. Manual sampling was performed every 12h.

AA methods

Cedex® Bio HT Analyzer from Roche Costum Biotech: Glutamine was analyzed from the manual samples only. The on-line mode was applied for glucose, lactate and ammonia quantification. HPLC: For on-line analysis of amino acids via HPLC an in-vial derivatization protocol was established. During a process the samples required dilutions between 1:64 and 1:8, which were included in the user defined programming (UDP) of the derivatization protocol. Aside from the in-needle derivatization the analytical method was performed as described by Hofer et al. (2017) [2].

Results

The HPLC could be successfully applied as monitoring tool for amino acids. A total of 19 amino acids were analyzed on-line. The analytical method was executed in 34 min, from which 10 min were needed for the addition of an internal standard, sample derivatization and dilution and 24 min corresponded to the actual run time for peak separation. Including the time needed for sampling, the amino acid values were available in Lucullus® after 45 min. In comparison, the Bio HT took 19 min for analysis, which corresponded to a total sampling and analysis time of 30 min until the data were available in Lucullus® PIMS. The modular structure of Numera® allows adaptation of the sample processing along with the analytical needs. Thus, filtration can be skipped for some Cedex® Bio HT analytes, which can further reduce sampling time. Moreover, a dilution can be included for the HPLC analysis if necessary (Table 1). Accuracy of the HPLC method was assessed in a CHO batch process. Figure 2A shows that online and off-line HPLC results were in good accordance, nicely representing the uptake of Gln over process time. The course of 17 other amino acids was monitored simultaneously. Furthermore, the results for Gln were compared between HPLC and Bio HT (Figure 2B). Both analyzers gave similar results which demonstrates the possibility to interchange them according to the analytical needs.

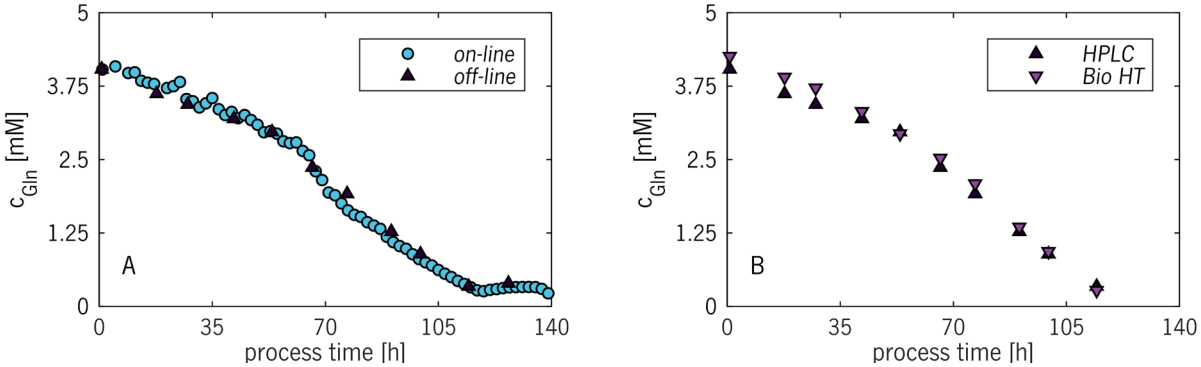

Figure 2: Plot A shows glutamine measured by HPLC on-line (light blue circles) and off-line (black triangles). Both measurements are in good accordance and nicely resemble the concentration course over process time. Plot B compares the analysis of the off-line samples via HPLC and Bio HT. Both show very similar results and hence, are interchangeable as on-line tools for amino acid analysis.

Moreover, the on-line HPLC was applied in a fed-batch CHO process monitoring 18 amino acids in parallel. Figure 3 illustrates the courses of three different amino acids over process time. Alanine concentration increased over process time, glutamine and asparagine decreased over process time. The latter two ones seem to be limiting. It should be noted that Gln was fed to the process starting after 160h.

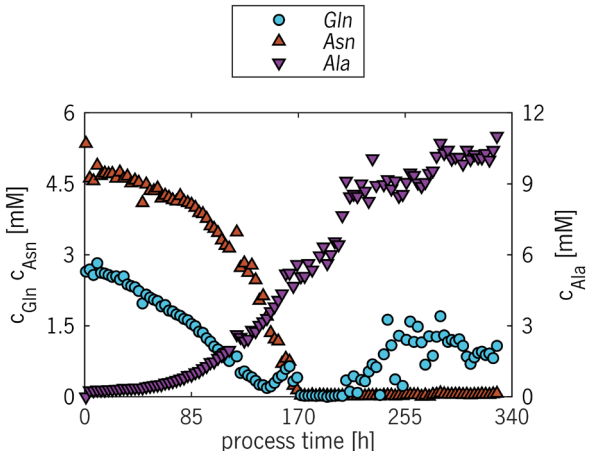

Figure 3: Different amino acids show different behavior during a fed-batch process. Glutamine (light blue circles) is a known limiting substrate; thus, feeding was started after 160h. Asparagine (red triangles) also showed limiting behavior but was not fed to the process. On the other hand, alanine concentration (violet upside-down triangles) increased over process time.

The on-line availability of these data allows faster generation of process understanding – e.g. concerning critical substrates. Additionally, the control strategy for Gln could be optimized using the analytical data available during the process.

Conclusion

The combination of Numera® and Lucullus® PIMS facilitates on-line availability of amino acid data from different analyzers such as HPLC or assay-based methods. Both presented methods are suitable for monitoring and show different features that have to be evaluated for the correct method choice for individual applications. The HPLC allows analysis of many amino acids, even in very low concentrations. The Cedex® Bio HT is limited in these two points but allows lower sampling intervals and is easier in handling. Different requirements for the sample can be met by Numera® as the routing module allows adapted sample processing, e.g. regarding dilution or filtration steps.

In summary, the combination of Numera® and a device for amino acid analysis is a powerful tool for bioprocess development aiming at generating process understanding and facilitating process control.

Key Results

– Automated sampling with Numera®
– Adaptation of sample processing to analytical needs (filtration, dilution)
– Numera® enables on-line monitoring
– HPLC and Cedex® Bio HT Analyzer are useful tools for on-line AA analysis

References

[1] Dionex Cooperation (now part of Thermo Fisher Scientific) (2016). "Automated In-Needle Derivatization Applying a User-Defined Program for the Thermo Scientific Dionex WPS-3000 Split-Loop Autosampler", Technical Note 107
[2] Hofer et al. (2018). "Prediction of filamentous process performance attributes by CSL quality assessment using mid-infrared spectroscopy and chemometrics", Journal of Biotechnology, 265, 93-100

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