AMINO ACID ANALYSIS: new challenges for an old technique?

Amino Acid Analysis Group, Large Molecule / ATMP Services, Solvias AG

Contact: aminoacidanalysis@solvias.com

Amino acid analysis is a chromatographic technique that was automated in the 1950s (1) and became widely used through amino acid analyzers (cation exchange chromatography with ninhydrin post-column derivatization). Mostly combined with acid hydrolysis, the determination of the amino acid ratio was an essential part of the structural elucidation of purified proteins. Further fields of application with or without hydrolysis are e.g. food chemistry, microbiology and clinical diagnostics.

After mass spectrometry replaced amino acid analysis in the structure elucidation of proteins, the determination of absolute protein content became a main focus of this method, since amino acid analysis is the orthogonal technique for all UV-based content determinations [2]. Under optimized hydrolysis conditions followed by an appropriate separation method and derivatization, 100% recovery is achieved for most amino acids with stable side chains meaning that the calculated protein content is independent of the number of aromatic and UV active side chains in the protein.

In the 1980s and 1990s, as well as around the turn of the millennium, further separation and derivatization methods for amino acids were developed. These methods can not only be used with amino acid analyzers but also on classic HPLC systems or UPLC instruments, in some cases in high-throughput mode.

In the pharmaceutical environment, amino acid analysis is used over a long period of time, primarily to determine the identity and content of synthetic peptides in quality control, even when containing non-proteinogenic amino acids. Robustness of analytical methods is an indispensable requirement under Q-standard GMP, so post-column derivatization with ninhydrin was and still is the best method for a large variety of peptides (1). When biologics and later biosimilars entered the market, new analytical requirements arose for amino acid analysis:

- Characterization of mAbs (e.g. extinction coefficients)
- content of excipients (buffer substances e.g. His, Arg, Met, Lys)
- in-process controls (e.g. content of cysteines as reducing agents)
- raw material analyses (almost all amino acids used as starting material for syntheses and formulations).

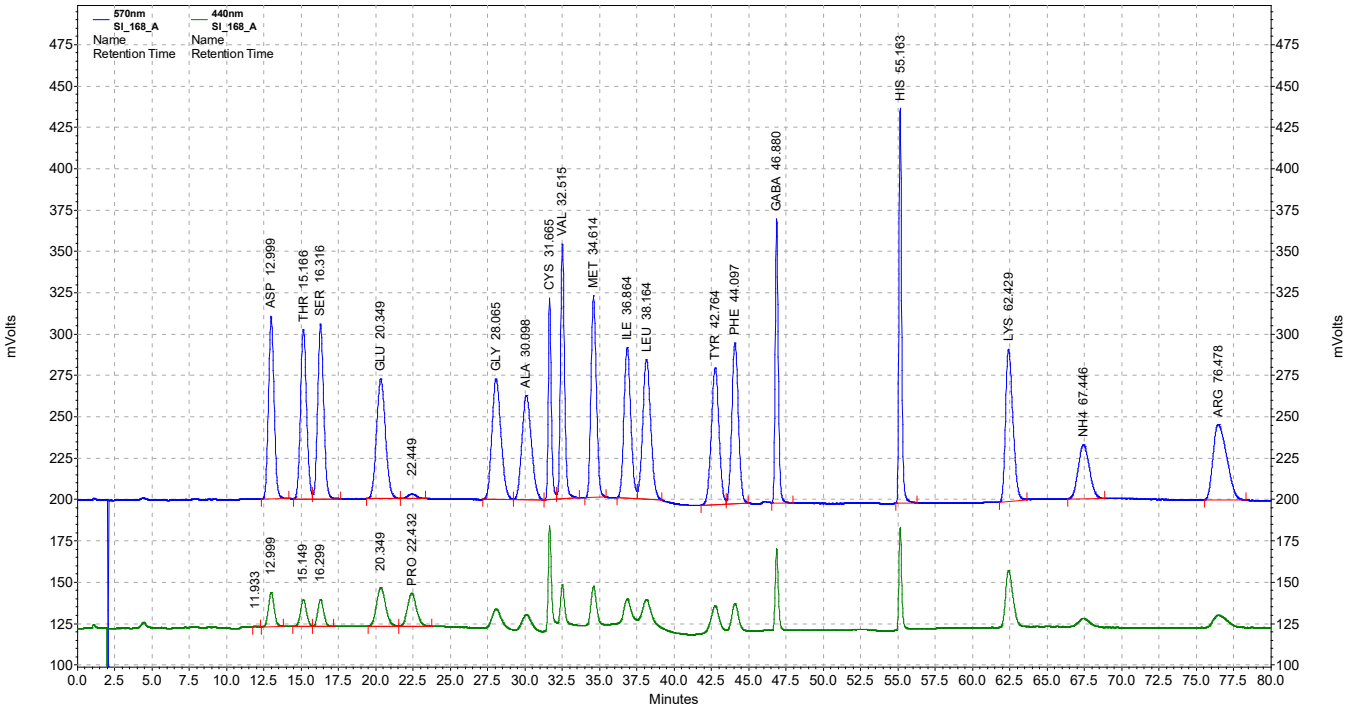

Figure 1: Chromatogram Hydrolysate

In the new ATMP/C&GT environment, regulatory requirements for the purity of raw materials (both single amino acids and complex media) have increased again, including the determination of amino acids in cell culture media for the growth of genetically modified patient cells.

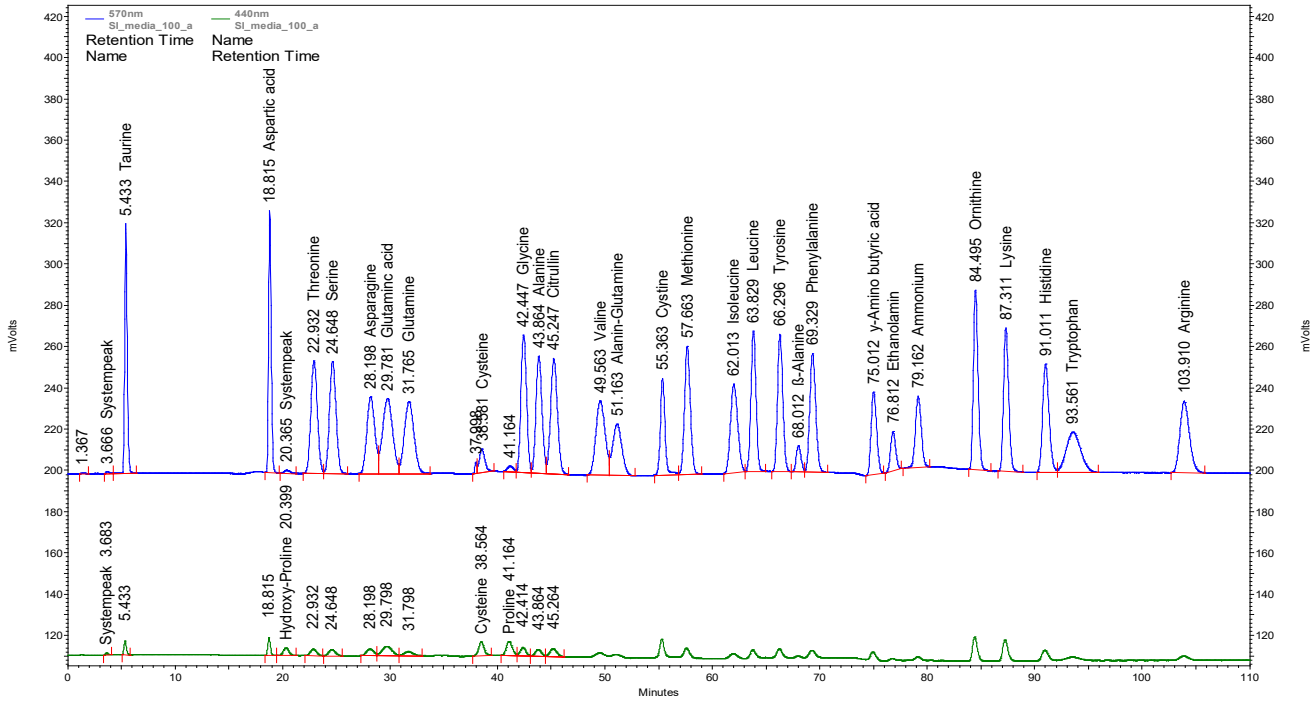

Figure 2: Chromatogram cell culture media

Testing of ninhydrin-positive substances in the amino acid monographs of Ph.Eur. has been successively changed over the last 10 years from thin-layer chromatographic to amino acid analysis (post-column derivatization with ninhydrin on the physiological system [3]). Using the amino acid analyzers, the whole range of all relevant impurities can be ideally covered from the acidic to the basic whereby ensuring a LOQ of at least 0.05% (w/w) - a robust quantification of impurities with high sensitivity is in the interest of patient safety.

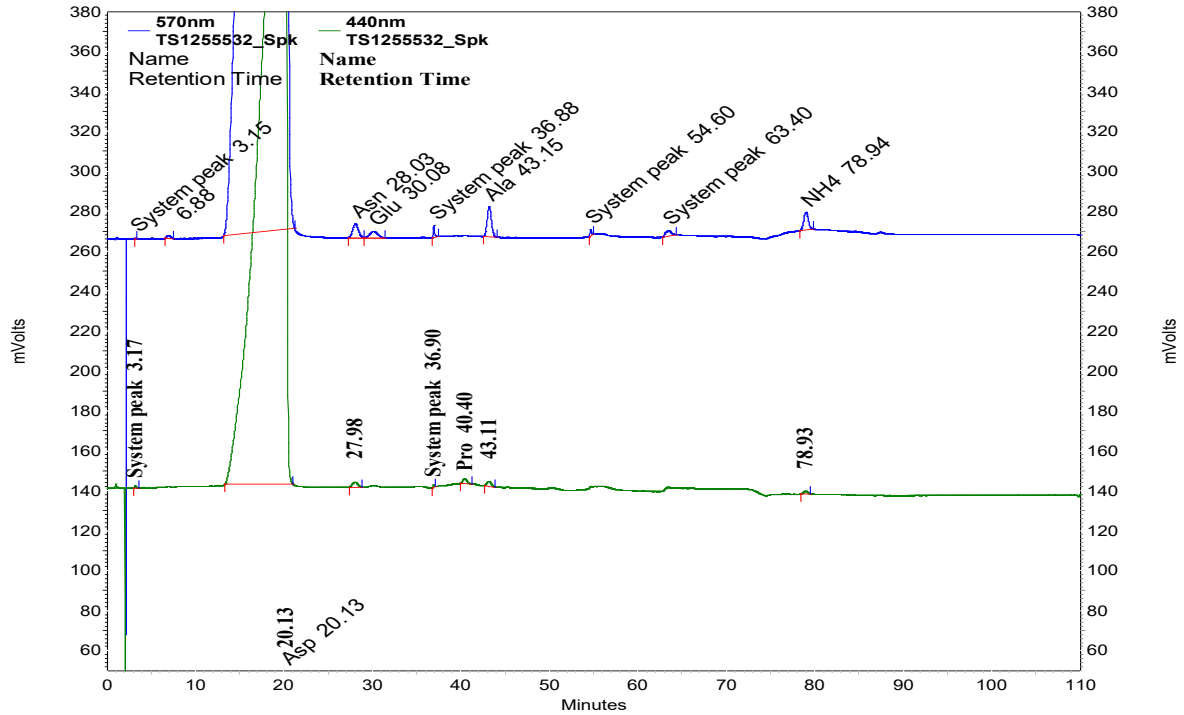

Figure 3: Chromatogram Raw material

Amino acid analysis at Solvias has an extensive method portfolio to cover the different needs of its customers:

- analysis of protein or peptide hydrolysates (UV-independent content determination)
- determination of excipients in complex matrices
- complete analysis of cell culture media (29 amino acid compounds and ammonium); for more detailed info see https://www.linkedin.com/posts/solvias-ag_aaa-in-cell-culture-media-activity-7087340307125563393-LCFV?
- Raw material analysis according to Ph.Eur. Monograph for 19 amino acids (test for ninhydrin-positive substances)

AMINO ACID ANALYSIS: new challenges for an old technique? That can be answered with an unqualified "yes".

[1] D.H. Spackman, W.H. Stein, S. Moore, Anal. Chem. 30 (1958) 1190

[2] M. Fountoulakis, H.-W. Lahm, J. Chrom. A, 826 (1998) 109-134

[3] Ph. Eur. 11.2., 01/2010: 20256, Amino acid analysis, Chromatography method 1