A METHOD FOR THE QUALITATIVE AND QUANTITATIVE DETERMINATION OF THE AMINO ACID COMPOSITION OF PHARMACEUTICAL PRODUCTS

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The United States Pharmacopeial Convention (USP), European Pharmacopoeia (Ph. Eur.) and Japanese Pharmacopoeia (JP) define requirements for the qualitative and quantitative composition of medicines. These pharmacopeias also outline the analytical methods to be carried out on pharmaceutical products as well as the substances and materials used in their production.

Amino Acid Analysis in general can be used for identification testing of biopharmaceutical active ingredients and the determination of impurities and related substances in active pharmaceutical ingredients. It can also be used for single or total amino acid quantitation in drug products. Our objective was to develop a liquid chromatography method for simultaneous determination of approximately 20 amino acids in simple and complex mixtures that comply with the system suitability requirements of the Ph. Eur. general chapter (2.2.56).

APPLICATIONS

For method development, the working standard contained 17 different amino acids at concentration 25 pmol/µL (Agilent Technologies) was used to evaluate separation and reproducibility. The program was optimized to have resolution between Leucine and Isoleucine at least 1.5. as per Ph. Eur. general chapter 2.2.56 requirements. Also, ammonia standard (0.12 ppm or 0.02% relatively to the sample concentration) was run at the same time to show that peak does not interfere with other peaks and have sufficient peak area counts to be calculate precisely from run to run. Ph. Eur. monographs for Arginine and Histidine (ninhydrin-positive substances and ammonium tests) were officially verified at our location. Several other monographs, including L-Lysine, L-Arginine, L-Glycine were successfully run in our lab.

Later on, HPLC method with post-column derivatization to test ninhydrin-positive substances and ammonium in L-Arginine per Ph. Eur. has been verified. Monograph listed L-Ornithine, L-Lysine and L-Citrulline as known impurities. Validation included Specificity, Linearity, LOQ, LOD, Precision, Intermediate Precision and Accuracy (spike-recovery). The method has been shown to be specific, linear, accurate and precise.

SGS Life Sciences (Chicago, IL) successfully applied this method to test amino acids present in complex formulations. For example, Arginine Hydrochloride serves as stabilizer for a biosimilar protein-based anti-inflammatory drug. The high-molecular weight API was filtered out and a low-molecular weight fraction was diluted and injected for analysis. We demonstrated excellent linearity (correlation 0.9997 at range 0.5-100 µg/mL), good method precision (%RSDn=5 = 0.2-0.6%) and acceptable accuracy across the 75-125% range (recovery within 96-121%) when performed spike-recovery study using Placebo.

At last, method is being used routinely to analyze amino acid composition of small proteins and polypeptides. Sample is hydrolyzed with 6N HCl at 110˚C for at least 20 hours and then injected. Individual amino acids are identified and quantitated against known standards. This test serves as an identification test and is based on individual amino acid relative composition (relative proportion of each amino acid to the total amino acid present in the sample).

METHOD

An HPLC System with a solvent delivery system, an autosampler, a dual wavelength detector and data collection module (Waters) were used. The method utilized a mobile phase gradient program as well as a column temperature gradient program for separation of amino acids of interest. After separation, eluent underwent post-column ninhydrin derivatization with subsequent detection at 570 nm and 440 nm for amino and imino acids, respectively (Pinnacle PCX Post-Column Derivatization Instrument). The HPLC parameters are outlined in Table 1 and the mobile phase gradient program is presented in Table 2. Table 3 illustrates the post-column derivatization system parameters and the column temperature gradient program is presented in Table 4.

The standards and test sample were injected into an HPLC with a sodium-based column (Pickering Labs Sodium Exchange, 4.6 × 110 mm, 5 µm). The samples were separated by a mobile phase gradient program coupled with a temperature gradient program to optimize amino acid separation. The column eluent underwent post column derivatization with ninhydrin. Ninhydrin-positive substances based on amino acids were detected at 570 nm; ninhydrin-positive substances based on imino acids, such as proline were detected at 440 nm.

TABLE 1 HPLC PARAMETERS

HPLC PARAMETERS	SETTING/DESCRIPTION
System	HPLC equipped with dual UV/Vis detector
Column	Pickering Labs Sodium Exchange, 4.6 × 110 mm, 5 µm
Detection	570 nm (Channel 1) 440 nm (Channel 2)
Flow Rate	0.6 mL/min
Injection Volume	50 µL
Column Temperature	gradient
Mode of Analysis	gradient
Run Time	45 min

TABLE 2: MOBILE PHASE GRADIENT PROGRAM

TIME (MIN)	MOBILE PHASE			
	%A NA315	%B NA425	%C NA640	%D RG011
0.0	100	0	0	0
4.0	100	0	0	0
20.0	0	100	0	0
21.0	0	0	100	0
38.0	0	0	100	0
38.1	0	0	0	100
39.9	0	0	0	100
40.0	100	0	0	0

TABLE 3: POST COLUMN DERIVATIZATION PARAMETERS

DERIVATIZATION SYSTEM PARAMETERS	SETTING/DESCRIPTION
System	Pinnacle PCX Pickering Laboratories
Reactor Temperature	130˚C
Reactor Volume	0.5 mL
Pump Rate	0.3 mL/min
Reagent	Trione®
Run Time	45 min
Washing Solution	20% Methanol
Mode of Analysis	gradient
Run Time	45 min

TABLE 4: COLUMN TEMPERATURE GRADIENT PROGRAM

TIME (MIN)	COLUMN TEMPERATURE ˚C
0.0	42
4.0	42
9.0	70
33.0	70
36.0	42

RESULTS

Using the Ph. Eur. general chapter (2.2.56) method, we were able to separate and identify the amino acids. No blank interference was observed at the retention time of amino acids of interest, indicating the specificity of the method (Figure 1). Blank peaks due to the mobile phase gradient were assigned based on blank injection. Likewise, the ammonium standard gave a single peak (Figure 2). The amino acids in the standard mix demonstrated good separation and were easily identifiable (Figure 3), as did the hydrolysed test sample (Figure 4). Proline, which is an imino acid, was the only peak detected at 440 nm (Figure 5).

The assay was tested for suitability with a set of select amino acids (Table 5). The %RSD of the working standard (instrument precision) for amino acids (~1.2 µg/mL or 0.2% to the sample concentration for analysis of individual amino acids per Ph. Eur. monographs) was not more than 15%. The %RSD for ammonium peak (0.12 ppm or 0.02% to the sample concentration) was not more than 15%. Resolution between Leucine and Isoleucine was not less than 1.5. Peak symmetry for amino acids and ammonium peak was 0.8-1.5. The method has been shown to be linear for selected amino acids in the range of 0.3-1.5 µg/mL (Table 6). The LOQ of the method for several amino acids was ~0.3 µg/mL or 0.05% relative to the sample concentration; whereas, the LOD of the method was ~0.1 µg/mL or 0.015% relative to the sample concentration.
Table 5. System Suitability

FIGURE 1: BLANK

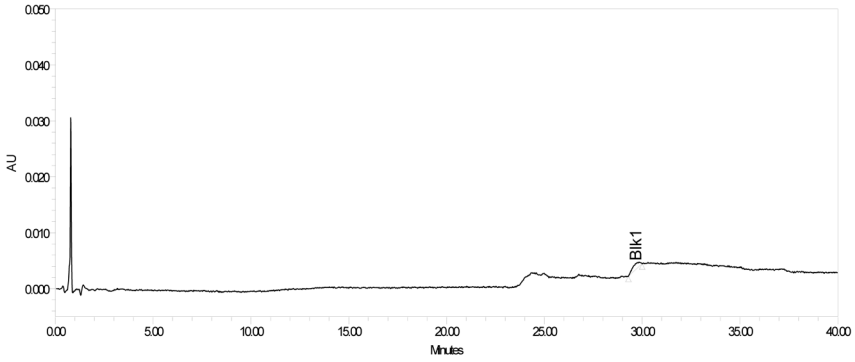

FIGURE 2: AMMONIUM STANDARD

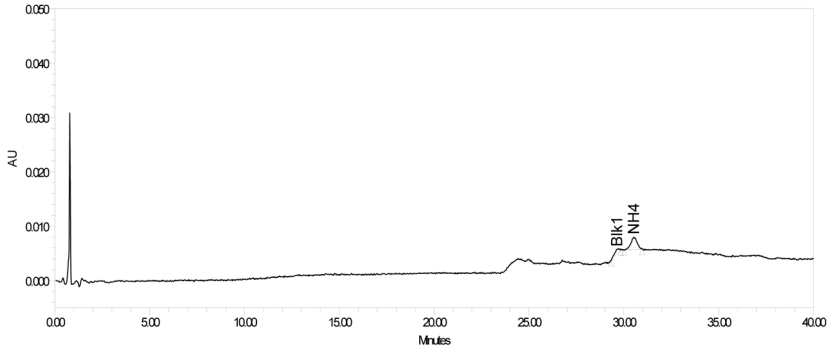

FIGURE 3: STANDARD

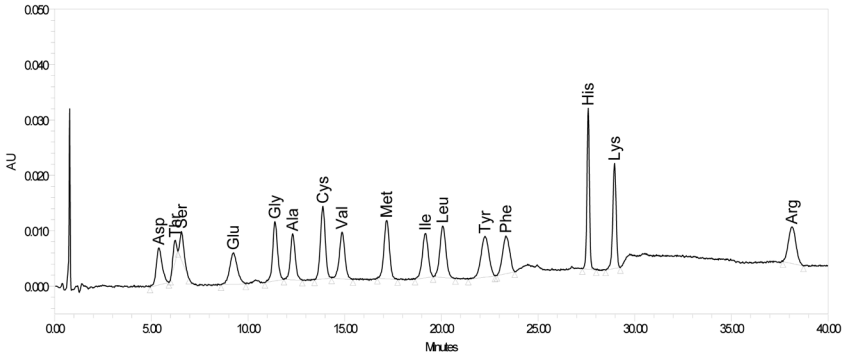

FIGURE 4: HYDROLYZED TEST SAMPLE

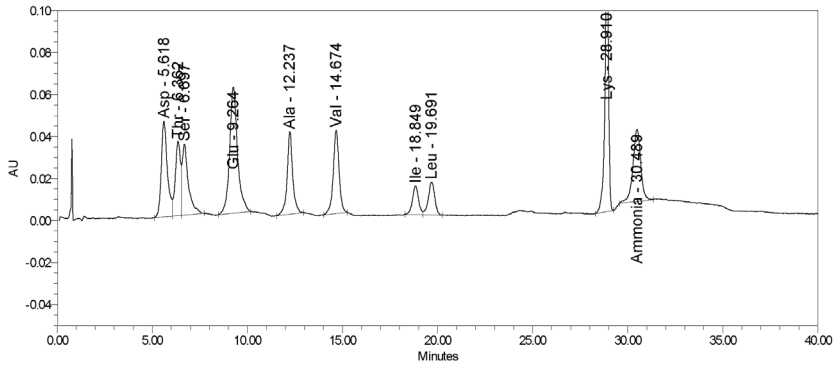

FIGURE 5: PROLINE (440 nm)

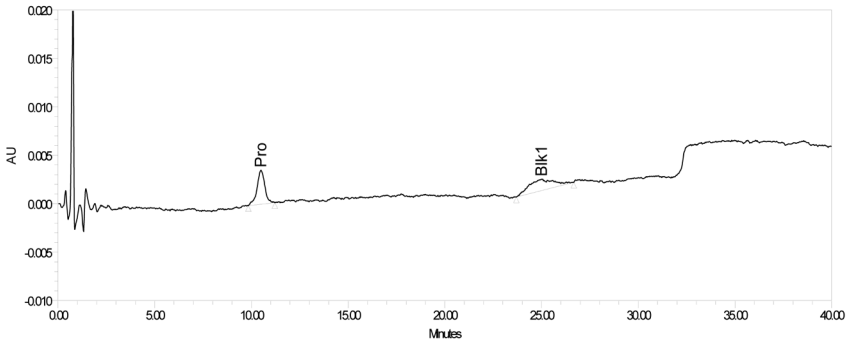

TABLE 5: SYSTEM SUITABILITY

ANALYTE	%RSD			
	RETENTION TIME	PEAK AREA COUNTS	PEAK HEIGHT	PEAK SYMMETRY
Alanine	0.1	1.3	1.9	1.1
Arginine	0.1	3.4	1.2	1.1
Aspartic Acid	0.3	2.4	2.3	1.1
Cystine	0.0	1.1	0.8	1.0
Glutamic Acid	0.2	4.5	1.6	1.0
Glycine	0.1	1.6	1.5	1.0
Histidine	0.0	0.8	0.8	1.0
Isoleucine	0.0	2.0	1.6	0.9
Leucine	0.0	1.2	1.1	1.1
Lysine	0.0	1.9	0.7	0.9
Methionine	0.1	2.1	1.6	1.0
Phenylalanine	0.1	1.2	1.2	1.0
Serine*	0.2	1.3	1.0	1.6
Threonine	0.1	6.0	6.6	0.8
Tyrosine	0.1	1.4	1.3	0.9
Valine	0.0	2.8	1.6	1.1
Ammonia	0.1	1.1	5.8	0.9
Proline	0.1	5.7	not evaluated	
Citrulline (at ~0.3 µg/mL)	0.1	6.1	not evaluated	
Ornithine (at ~0.3 µg/mL)	0.0	1.2	not evaluated	

**Valley-to-valley integration was applied.*

TABLE 6: LINEARITY

AMINO ACID 0.3-1.5 µG/ML	CORRELATION COEFFICIENT (R)	SLOPE	Y-INTERCEPT
Citrulline	0.998267	1.300004E+005	-2.234584E+003
Ornithine	0.999781	2.225075E+005	-6.707581E+003
Lysine	0.999403	1.640153E+005	-8.985859E+003
Arginine	0.997937	1.185956E+005	-8.338416E+003
Arginine HCl (0.5-104 µg/mL)	0.999701	1.074797E+005	-5.615213E+004

CONCLUSIONS

The developed method was optimized to comply with system suitability requirements per the Ph. Eur. general chapter (2.2.56). Our laboratory has successfully verified the method for analysis of ninhydrin-positive substances in individual amino acid samples per the Ph. Eur. monographs. While this method has been used to evaluate excipients (amino acids) concentrations in generic and biosimilar drugs, the method has been used to evaluate relative amino acid composition in small proteins and peptides.

REFERENCES

Ph. Eur. 9.0 (2.2.56) Amino Acid Analysis
Ph. Eur. 8.0 Arginine Hydrochloride Monograph
USP 39 <1226> Verification of Compendial Procedures
Pickering Laboratories Method Abstract/391 Amino Acid Analysis according to European Pharmacopeia 8.0
ICH Q2 (R1) Validation of Analytical Procedures, Text and Methodology

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