VALIDATION OF AN UPLC METHOD FOR THE DETERMINATION OF AMINO ACIDS IN FEEDS

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An effective monitoring of different feed materials in the scope of amino acids involves using a rapid and specific method like a reverse phase ultra-performance liquid chromatography (UPLC). The aim of the present study was to validate an UPLC method for quantification of 17 amino acids (histidine, serine, arginine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, lysine, tyrosine, valine, isoleucine, leucine, phenyloalanine, cystine, methionine) in feeds, and to compare obtained results with performance characteristics of the official ion-exchange chromatographic method published in the regulation (EC) No 152/2009 [1]. Feeds used in the study were: rye grains, porcine hemoglobin and feed mixture, with different content of amino acids. Samples were hydrolyzed with hydrochloric acid, filtrated, evaporated and dissolved in water. The 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) was used as pre-column derivatizing reagent. Cystine and methionine were oxidized to derivatives – cysteic acid and methioninesulfone, respectively. Products of pre-column derivatisation were analyzed chromatografically with using Waters ACQUITY UPLC system. The reversed phase separation occurs on the AccQ·Tag Ultra column and detection at 260 nm. For each amino acid LOQ, repeatability, within-laboratory reproducibility, recovery and expanded uncertainty were determined. LOQ for analyzed amino acids were from 0.2 g/kg (for methionine) to 2.2 g/kg (for glutamic acid). The precision and accuracy parameters as well as uncertainty for some amino acids are summarized in the Table 1 below. Expanded uncertainty (k=2) was quantified as duplicate within-laboratory reproducibility (intermediate precision). Expanded uncertainties were also calculated using proficiency testing data and for selected amino acids amounted: 22% for methionine, 11% for threonine, 16% for lysine and 16% for cystine.

Table 1. Some performance parameters of the UPLC method compared to the official method for the chosen amino acids

Amino acid	Range, $g \cdot kg^{-1}$	UPLC, repeat.%	UPLC, int.prec.%	UPLC, Recovery,%	U (k=2) %	Repeat. 152/09,%	Reprod. 152/09,%
Thr	0.4-31.0	0.6-1.9	1.1-2.3	98.2-102.0	6.8-9.6	1.9-2.7	3.8-5.2
Lys	0.6-77.0	1.5-2.2	2.6-3.2	92.8-95.4	6.0-9.6	2.1-2.8	3.0-5.4
Cys	0.5-7.0	1.5-3.7	4.0-5.7	93.0-96.6	8.6-10.4	2.6-3.3	8.8-12.3
Met	0.2-9.0	0.8-1.9	2.2-6.8	87.1-97.0	8.4-10.4	2.2-3.4	7.0-13.0

Accuracy of the UPLC method was confirmed in the proficiency testing organized by AGES, Austria: IAG - Feedingstuffs 2014 and satisfying results were obtained (mean *z*-score equal to 0.37; from -1.1 to 0.7). The UPLC method is characterized by validation parameters similar to the official method and can be used for determination of amino acids in feeds as well as a method for official control purpose.

[1] Commission Regulation (EC) No 152/2009 – OJ L54/1, 26.2.2009