Estimation of uncertainty from method validation data: application to a reverse-phase high-performance liquid chromatography method for the determination of amino acids in gelatin using 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate reagent

ABSTRACT

A detailed procedure for estimating uncertainty according to the Laboratory of Government Chemists/Valid Analytical Measurement (LGC/VAM) protocol for determination of 18 amino acids in gelatin is proposed. The expanded uncertainty was estimated using mainly the method validation data (precision and trueness). Other sources of uncertainties were contributed by components in standard preparation measurements. The method scope covered a single matrix (gelatin) under a wide range of analyte concentrations. The uncertainty of method precision, μ(*P*) was 0.0237–0.1128 pmol μl^{-1} in which hydroxyproline and histidine represented the lowest and highest values of uncertainties, respectively. Proline and phenylalanine represented the lowest and highest uncertainties value for method recovery, μ(*R*) that was estimated within 0.0064–0.0995 pmol μl^{-1}. The uncertainties from other sources, μ(*Std*) were 0.0325, 0.0428 and 0.0413 pmol μl^{-1} that were contributed by hydroxyproline, other amino acids and cystine, respectively. Hydroxyproline and phenylalanine represented the lowest and highest values of expanded uncertainty, U(*y*) that were determined at 0.0949 and 0.2473 pmol μl^{-1}, respectively. The data were accurately defined and fulfill the technical requirements of ISO 17025:2005.

Keyword: Gelatin; Amino acids; 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate reagent; Estimation of uncertainty