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BOOK OF ABSTRACTS

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EVALUATION OF ENZYMATIC DIGESTION CONDITIONS FOR DETERMINATION OF IMMUNOGLOBULINS BY TANDEM MASS SPECTROMETRY

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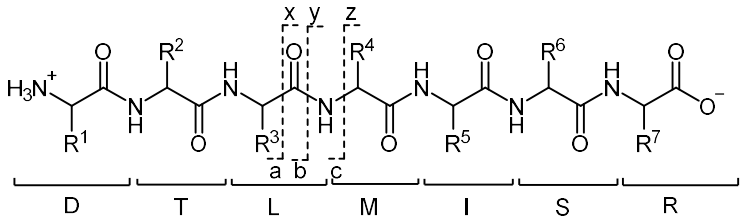
Immunoassays, namely ELISA, have been the standard method for detecting clinically significant immunoglobulins (Igs). They are based on Ig-antigen interaction, often suffering interference from matrix components. New analytical approaches using detection by tandem mass spectrometry (MS/MS) search for fundamental structure information of target Igs based on protein features. In fact, there are few examples of quantitative assays achieved by liquid chromatography coupled with triple quadrupole (QqQ) mass analyzers. Due to the limited mass range of QqQ, the use of this mass analyzer requires previous tryptic digestion of IgG for analysis of highly specific surrogate peptides. In this work, initial studies on a LC-MS/MS method for the quantitative analysis of IgG are reported. The method relies upon the detection of the generic peptide DTLMISR (Fig. 1), originated from the fraction crystallizable (Fc) region of IgG after enzymatic cleavage. The multiple reaction monitoring transitions used for quantification and identification purposes were, respectively, *m/z* 418.20 → 506.10 and 418.20 → 619.30, corresponding to the fragmentation of double-charged molecular ions. In order to investigate the influence of trypsin concentration on digestion kinetics and efficiency, the trypsin-to-protein ratios 1:20, 1:50 and 1:100 were evaluated. Moreover, the performance of the digestion process was monitored for IgG standards and plasma samples over 18 h at 37 °C. Using a 1:50 ratio, two distinct kinetic profiles were observed for standards and plasma samples with a maximum signal intensity after 6 and 18 h, respectively.



D = aspartic acid, $R^1 = CH_2COOH$
T = threonine, $R^2 = CH(OH)CH_3$
L = leucine, $R^3 = CH_2CH(CH_3)_2$
M = methionine, $R^4 = (CH_2)_2SCH_3$
I = isoleucine, $R^5 = CH(CH_3)CH_2CH_3$
S = serine, $R^6 = CH_2OH$
R = arginine, $R^7 = (CH_2)_3NHC{=}NH(NH_2)$

Figure 1: Structure of the peptide DTLMISR selected for quantification by LC-QqQ-MS/MS.

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