
COMPARISON OF REVERSE PHASE CHROMATOGRAPHY- AND HYDROPHILIC INTERACTION CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY IN THE STUDY AND CHARACTERIZATION OF O-SIALOGLYCOPEPTIDES FROM HYDROLYZED CASEINOMACROPEPTIDE

O. Hernández (1)*, R. Lebrón-Aguilar (2), J.E. Quintanilla-López (2, 3), M.L. Sanz (1) and F.J. Moreno (4)#

(1) Instituto de Química Orgánica General (CSIC), C/ Juan de la Cierva, 3 28006 Madrid, Spain, * oswaldohh@iqog.csic.es

(2) Instituto de Química-Física “Rocasolano” (CSIC), C/ Serrano, 119 28006 Madrid, Spain

(3) Departamento de Ingeniería Química Industrial y del Medio Ambiente, ETS Ingenieros Industriales (UPM) C/ José Gutiérrez Abascal, 2 28006 Madrid, Spain

(4) Instituto de Fermentaciones Industriales (CSIC), C/ Juan de la Cierva, 3 28006 Madrid, Spain, # j.moreno@ifi.csic.es

Proteins can be found in nature glycosylated with different carbohydrates by both *N*- and *O*- linkages. *O*-linked glycosylation consists of attaching the glycans to the hydroxyl oxygen of serine and threonine residues in sequence regions of high hydroxyamino acid density without a unique sequence motif required for this attachment. Considering the heterogeneity of both peptide and oligosaccharide structures for *O*-glycoproteins, their analysis is not trivial and advanced analytical techniques are required (1).

Different HPLC separation modes (i.e. size exclusion, anion exchange, reverse phase (RP), amine-bonded silica phases, etc) are commonly used to analyse glycoproteins. RPLC is the most extended among them, but in the last decade, hydrophilic interaction liquid chromatography (HILIC) is gaining a great importance in the analysis of glycopeptides. In recent times, *N*-glycopeptides have been widely analysed by LC-tandem mass spectrometry (MSⁿ) but little information is found about *O*-glycopeptides. Therefore, the aim of this work was to develop a HILIC-MSⁿ method to characterize *O*-sialoglycopeptides in a tryptic/chymotryptic hydrolyzate of bovine caseinomacropetide and to compare its efficiency with the RPLC-MSⁿ method. Behaviour of non-glycosylated peptides was also studied.

Peptides were separated on a zwitterionic HILIC column using an elution gradient of acetonitrile and water with 0.005% formic acid. For RPLC method a Hypersil HyPurity C18 column (Thermo Fisher Scientific) with a gradient of acetonitrile and water with 0.1% of formic acid was used. Detection for both chromatographic modes was carried out on an ion trap mass spectrometer in positive mode.

Different separation behaviour for both non-glycosylated peptides and *O*-glycopeptides by RPLC and HILIC was observed. Non-glycosylated peptides were more retained than those glycosylated by RPLC; however, although more complex chromatograms were obtained by HILIC, the separation achieved by this technique was better for the *O*-sialoglycopeptides. Therefore, the optimized method made the detection of peptides by MSⁿ easier.

LC-MS² analyses of *O*-glycopeptides mainly resulted in glycosidic bond fragmentation with the corresponding ion of the intact peptidic chain and the respective losses of carbohydrates units. To confirm the amino acidic sequences of glycopeptides, MS³ spectra were successfully performed from the ion corresponding to the intact peptidic chain. This method allowed the identification and characterization of ~30 *O*-glycopeptides.

In conclusion, HILIC-MSⁿ has shown to be a powerful technique for the identification of *O*-glycopeptides, allowing the detection of a higher number of compounds than RPLC-MSⁿ without any previous derivatization treatment.

(1) Seipert et al. (2009), Journal of Proteome Research, 8, 493-501.

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