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P-32

Determination of Amino Sugars in Blood Serum Samples by RP-HPLC Method

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1. Introduction

Amino sugars (aminosaccharides, hexosamines) represent an important group of compounds that are present in all living organisms as part of the structural units of some biopolymers and are also found in dead organic residues that pass into the structure of humic substances due to humification processes [1].

Structurally, amino sugars are derivatives of monosaccharides where one hydroxyl group (-OH) is substituted for an amino group ($-NH_2$). Like sugars, amino sugars are chiral compounds, free of chromophores and fluorophores. By comparing their structures, they represent stereoisomers, in particularly epimers, among themselves. Epimers are stereoisomers differing in configuration at one stereogenic center but in a different than the last asymmetric carbon atom and the anomeric carbon atom. It follows that the amino sugars glucosamine (GlcN) and galactosamine (GalN), differing in the orientation of the bond on the fourth carbon, are C-4 epimers and glucosamine with mannosamine (ManN) represent C-2 epimers. In addition to simple forms of amino sugars, N-acetylamino sugars are also present in most biopolymers, which are formed by acetylation of the primary amino group [2,3].

Among the amino sugars, glucosamine is the most widespread in chitin, the structural polymer of most invertebrates, fungi and algae. Along with another amino sugar, muramic acid (Mur) is found in peptidoglycans, a major component of bacterial cell walls [3]. Amino sugars are also found in tissues and body fluids of higher organisms, including humans. Their presence in synovial fluids suggests that amino sugars (especially GlcN) may be used in the symptomatic treatment of osteoarthritis [4], manifested by gradual degradation of the cartilage in the joints, possibly leading to complete cartilage loss.

Given the importance of amino sugars, not only in the understanding and treatment of osteoarthritis, there is a need to develop a sensitive and reliable analytical method for their determination in clinical samples. The main problem in the development of this method is the character of amino sugars, namely their high polarity and low concentrations (the content of GlcN in the blood is 60 $ng.mL^{-1}$) [5,6]. The presented work was focused on separation of three amino sugars (GlcN, GalN, ManN) by RP-HPLC method with pre-column derivatization of analytes using diethylethoxymethylenemalonate reagent (DEEMM). The developed method was subsequently applied for the determination of analytes in a blood serum sample.

3. Results

Due to the natural occurrence of amino sugars in two anomeric forms (α- and β-anomer), it can be assumed that both isomers are also present in aqueous solutions. Based on the chromatographic

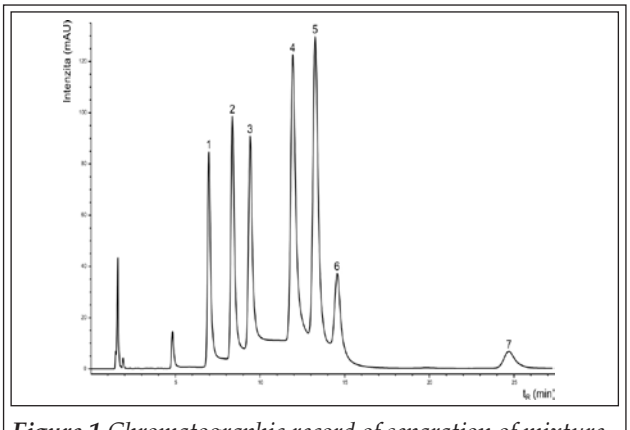

Figure 1 *Chromatographic record of separation of mixture of amino sugars standards at 280 nm (1 - ManN 1, 2 - GalN 1, 3 - GlcN 1, 4 - GalN 2, 5 - GlcN 2, 6 - ManN 2, 7 – DEEMM)*

Table 1 *Calculated detection and quantification limits for individual amino sugars*

Amino sugar	LOD (mg.L^{-1})	LOQ (mg.L^{-1})	LOD (μmol.L^{-1})	LOQ (μmol.L^{-1})
ManN 1	0,033	0,099	0,153	0,459
GalN 1	0,039	0,120	0,181	0,557
GlcN 1	0,039	0,120	0,181	0,557
GalN 2	0,032	0,097	0,148	0,450
GlcN 2	0,026	0,080	0,121	0,371
ManN 2	0,084	0,256	0,390	1,187

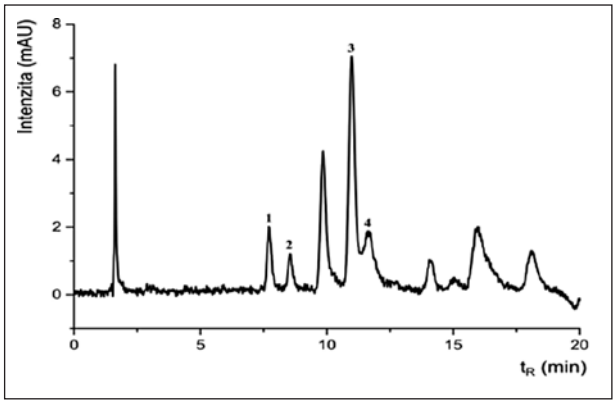

Figure 2 *Chromatographic record of blood serum sample at 280 nm (1 - GalN 1,2, - GlcN 1,3, - GalN 2,4, - GalN 2)*

record of separation of the mixture of amino sugar standards (***Figure 1***), we can confirm that the assumption of both forms of amino sugars in the solution has been confirmed.

After optimizing separation of the mixture of amino sugar standards, we measured the 6-point calibration curves of the individual amino sugar anomers, at concentration intervals 0.05-5.0 mg.L-1 (0.05; 0.1; 0.5; 1.0; 2.0; 5.0 mg.L^{-1}), with five repetition of all concentrations. From the calibration data, we calculated the detection limit (LOD), defined as the signal-to-noise ratio (S/N = 3:1) and the determination limit (LOQ) (S/N = 10:1) of the individual amino sugar anomers (Table I).

The blood serum sample supplied by the Faculty of Medicine of Comenius University in Bratislava after derivatization by DEEM reagent was analyzed by RP-HPLC method (***Figure 2***). In the blood serum sample, we determined two amino sugars, glucosamine and galactosamine, using the standard addition method, at concentration levels of 1.034-3.343 mg.L^{-1}.

4. Conclusions

The work deals with separation of the three most important amino sugars (glucosamine, galactosamine, mannosamine) by RP-HPLC method. The developed method was applied for the determination of analytes in a blood serum sample in which we determined the anomers of two selected amino sugars at concentration levels of 1.034 - 3.343 mg.L^{-1}. An advantage of the method is also the use of formate buffer, which is a suitable mobile phase also for the combination of liquid chromatography mass spectrometry with electrospray ionization (LC-ESI-MS or LC-ESI-MS / MS). The presented RP-HPLC method can be applied to other clinical samples (synovial fluid, cerebrospinal fluid, blood plasma) as well as environmental samples (water, soil, humic acid degradation products).

5. Acknowledgements

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References

1. *Coelho, R.R.R., Sacramento, D.R.: Amino sugars in fungal melanins and soil humic acids, Eur. J. Soil. Sci., 48: 425-429 (1997).*
2. *Gooday, G.W.: The Ecology of Chitin Degradation, Adv. Microb. Ecol., 11: 387-430 (1990).*
3. *Niggemann, J., Schubert, C.J.: Sources and fate of amino sugars in coastal Peruvian sediments, Geochim. Cosmochim. Acta, 702229-2237 (2006).*
4. *Zhang, L., Huang, T., Fang, X., Li, X., Wang, Q., Zhang, Z., Sha, X.: Determination of glucosamine sulfate in human plasma by precolumn derivatization using high performance liquid chromatography with fluorescence detection: Its application to a bioequivalence study, J. Chromatogr. B, 842: 8-12 (2006).*
5. *Pastorini, E., Rotini, R., Guardigli, M., Vecchiotti, S., Persiani, S., Trisolino, G., Antonioli, D., Rovati, L.C., Roda, A.: Development and validation of a HPLC-ES-MS/MS method for the determination of glucosamine in human synovial fluid, J. Pharmaceut. Biomed., 50: 1009-1014 (2009).*
6. *Hubert, C., Houari, S., Lecomte, F., Houbart, V., De Bleye, C., Fillet, M., Piel, G., Roset, E., Hubert, P.: Development and validation of a sensitive solid phase extraction/hydrophilic interaction liquid chromatography/mass spectrometry method for the accurate determination of glucosamine in dog plasma, J. Chromatogr. A, 1217: 3275-3281 (2010).*