Methods of determination of pentosidine – the advanced glycation end-product of proteins

AGNIESZKA NOGAJCZYK[1], MAGDALENA SZUMSKA[1], BARTŁOMIEJ KUMASZKA[2], KRYSTYNA TYRPIEŃ-GOLDER[1]

[1]KATEDRA I ZAKŁAD CHEMII, WYDZIAŁ LEKARSKI Z ODDZIAŁEM LEKARSKO-DENTYSTYCZNYM W ZABRZU, ŚLĄSKI UNIWERSYTET MEDYCZNY W KATOWICACH

[2] SZPITAL POWIATOWY IM. PRAŁATA J. GŁOWATZKIEGO W STRZELCACH OPOLSKICH

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ABSTRACT

Glycation is a multi-step process that occurs spontaneously without the presence of enzyme, leading to the formation of AGEs – Advanced Glycation End-products. Glycation occurs in living organisms as well as in the food under the influence of heat treatment and long-term storage. In a healthy body the majority of AGE is metabolized and excreted. With time, some amounts of these compounds accumulate in tissues resulting in the development of many chronic diseases.

One of AGEs is pentosidine. It is formed by reaction of lysine and/or arginine with reducing sugar.

The aim of the study was to collect information on pentosidine and to present a review of already applied and the latest methods of its determinations in biological samples.

Studies described in literature provide information on the quantification of pentosidine by: immunoassay (ELISA), high performance liquid chromatography (HPLC) mostly coupled with various detectors. However, the opinions of researchers are divided regarding choosing the best method of pentosidine analysis. Pentosidine has been so far determined in the blood, urine, saliva, bone, skin tissue, articular cartilage. It plays an important role in the etiopathogenesis of many diseases (diabetes, kidney damage, rheumatoid arthritis, osteoarthritis, asthma). The concentrations of pentosidine in the blood of healthy individuals, described in the literature range from 0.21 pmol to 1.4 pmol/mg protein and in the case of patients suffering from the disease mentioned above it can reach the concentration up to 27.3 pmol/mg protein.

Metody oznaczania pentozydyny – produktu zaawansowanej glikacji białek

Słowa kluczowe: pentozydyna, wysokosprawna chromatografia cieczowa, techniki immunoenzymatyczne

STRESZCZENIE

Glikacja to wieloetapowy proces, który zachodzi spontanicznie bez udziału enzymów i prowadzi do powstawania AGEs (Advanced Glycation End-products), czyli zaawansowanych (końcowych) produktów glikacji. Proces ten zachodzi w żywych organizmach, a także w żywności pod wpływem obróbki cieplnej oraz długotrwałego i nieprawidłowego jej przechowywania. W zdrowym organizmie większość AGE jest metabolizowanych i wydalanych. Z czasem jednak pewne ilości tych związków odkładają się w tkankach, powodując rozwój przewlekłych schorzeń.

Jednym z końcowych produktów glikacji jest pentozydyna. Powstaje ona w wyniku reakcji lizyny i/lub argininy z cukrem redukującym.

Celem pracy było zebranie informacji na temat pentozydyny oraz przedstawienie przeglądu stosowanych dotychczas i najnowszych metod jej oznaczeń w próbkach biologicznych.

W piśmiennictwie można znaleźć informacje na temat ilościowego oznaczania pentozydyny za pomocą: testu immunoenzymatycznego ELISA (enzyme-linked immunosorbent assay) i wysokosprawnej chromatografii cieczowej HPLC z różnymi detektorami. Jednak zdania autorów co do wyboru najlepszej metody są podzielone. Pentozydyna oznaczana jest głównie we krwi, moczu, ślinie, kościach, skórze i chrząstce stawowej. Odgrywa ona ważną rolę w etiopatogenezie wielu jednostek chorobowych (cukrzyca, uszkodzenia nerek, reumatoidalne zapalenie stawów, zwyrodnienia stawów, astma). Stężenia pentozydyny we krwi osób zdrowych, oznaczone technikami immunochemicznymi lub chromatograficznymi, opisane w dostępnym piśmiennictwie, mieszczą się, w zależności od metody analizy, w zakresie od 0,21 pmola do 1,4 pmola w przeliczeniu na mg białka, natomiast w przypadku wymienionych jednostek chorobowych osiągają wartości nawet do 27,3 pmol/mg białka.

1. STAGES OF PROTEINS GLYCATION AND ITS EFFECT ON AN ORGANISM

The term glycation mainly refers to non-enzymatic binding of sugars to proteins. This description made it possible to distinguish this process from the enzymatic glycosylation.

The essence of this reaction is the formation of a covalent bond between the carbonyl group or hemiacetal group of reducing sugars and the free amino group of proteins, amino acids, peptides, phospholipids or nucleic acids [1]. Glycation is a spontaneous reaction that occurs in the living organisms, as well as in the food due to the heat treatment and the long-term storage. The reaction between sugars and amino acids was first described in 1912 by Louis Maillard (Fig. 1) [2].

The process of glycation is a multistep one which rate depends, inter alia, on the concentration of the reactants and time of their reaction. The first step is a reversible reaction of the carbonyl group of sugar with a primary amino group, followed by elimination of water and formation of Schiff base (aldimine). This process reaches an equilibrium state within a few hours. The formed compound undergoes the rearrangement to form Amadori 1-amino-1-deoxyketose (PPA – Amadori rearrangement product). It may form the conformation of the cyclic pyranose or furanose. This reaction reaches the equilibrium stage approximately after 28 days. As the result of the described transformations so called early glycation products are formed. If the reaction concerns proteins of long half-life the further chemical transformations such as oxidation, dehydration, fragmentation and condensation of other amino groups take place, which may last for several months or even years [3].

The Maillard reaction may also take place in the presence of oxygen and in this case PPA conversions may occur, which result in the formation of 1- and 3-deoxydicarbonyl compounds. During these transformations the reactions of dehydration, oxidation can occur and numerous transformations leading to the release of reactive oxygen species (ROS), organic radicals, and dicarbonyl

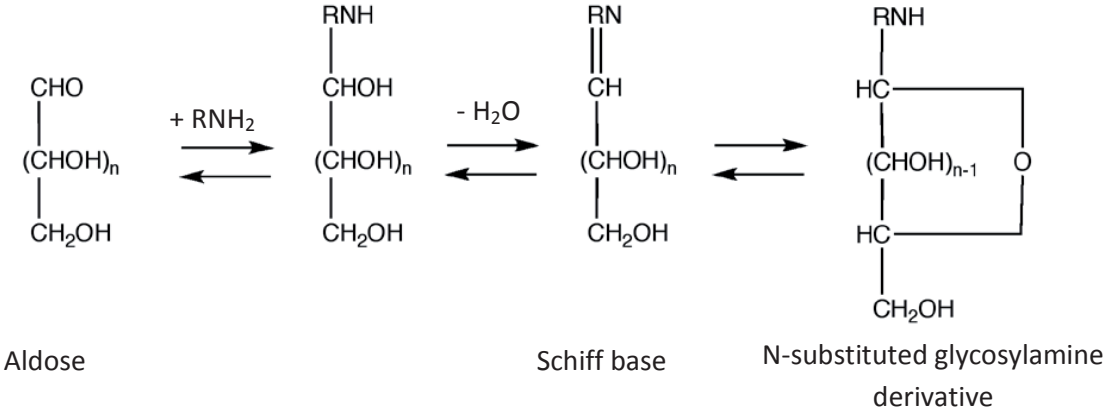

Figure 1 The fundamental equation of Maillard reaction [2]

compounds such as glyoxal, methylglyoxal, 3-deoxyglucosone [1]. The resulting compounds are highly reactive and therefore they react with amino groups of proteins to form advanced glycation end-products AGEs. These compounds may bind with each other and with some proteins, forming cross-bonds, thereby disrupting the activity of most cells and tissues of the body [4].

Glycation end-products may accumulate in organs and tissues, which in turn leads to serious diseases such as: osteoarthritis, osteoporosis and many neurodegenerative diseases [6-10].

Some proteins that undergo glycation may lose their original function. In case of albumin, this process leads to reduction of its ability to transport bilirubin and long chain fatty acids. Erythrocyte membrane protein loses its elastic properties and its deformation ability in the capillaries. Glycated cathepsin B shows reduced activity which leads to disturbances in the conversion of proinsulin to insulin. As a result of condensation of antithrombin III with reducing sugars the processes of coagulation prevail over fibrinolysis. Furthermore, glycation inhibits the activity of superoxide dismutase, which contributes to the formation of oxidative stress.

Due to glycation processes of collagen, cross-linking of the tissue structure occurs and cross-bonds are formed resulting in the increased stiffness of the collagen fibers, reduced solubility of fibers as well as their susceptibility to enzymatic digestion and collagen regeneration. The nucleic acids are characterized by long half-life and may also undergo glycation which, in turn, leads to changes in the genetic material [8].

The aim of the study was to collect information on pentosidine – one of advanced glycation products, and to provide a review of already applied the latest methods of its determinations in biological samples.

2. PENTOSIDINE – A PRODUCT OF AGES ADVANCED STAGE OF THE MAILLARD REACTION

Pentosidine is one of the advanced glycation end-products. It is formed in the reaction between lysine, arginine and reducing sugar. Besides pentoses, glucose also can be the substrate for its formation [8]. Pentosidine, for the first time, was described in 1989 by Sell and Monnier, who isolate it from the meninges [5]. This compound has also been identified in thermally processed food for example in roasted coffee, bakery and dairy products [2, 11]. In the tissues the concentration of pentosidine increases linearly with age. Its concentration depends on the transformation processes of collagen – its high concentration is observed in tissues characterized by a very slow changes of collagen structure (e.g. trachea), and lower in tissues with a faster transformation of collagen (e.g. dura mater, aorta, renal medulla, skin) [8].

In the human body predominates protein-bound form of pentosidine formed by arginine cross-linked with lysine in the presence of carbonyl derivatives. Free pentosidine, which molecular weight is 379 (molecular formula $C_{17}H_{27}N_6O_4$), is composed of a imidazole-pyridine ring, with side chains of lysine and arginine (Fig. 2), and it is formed by hydrolysis of the protein [5].

Sell and Monnier [12] found that the absorbance of UV radiation by pentosidine occurs at a wavelength of 320 nm and it is pH-dependent. The maximum absorbance is observed at pH 12 and

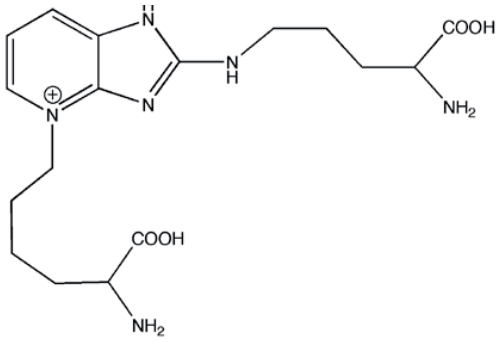

Figure 2 Structure of free pentosidine [5]

minimum at pH 2. Molar absorptivity of synthetic and native pentosidine, dissolved in 0.1 M HCl, equals 4195 and 4522 AU [$dm^3 \times mol^{-1} \times cm^{-1}$] respectively. This compound shows maximum fluorescence at the excitation wavelength of 335 nm and emission – 385 nm. The fluorescence intensity is also pH-dependent and its maximum is observed at pH = 2, and the minimum at pH = 9 [12].

3. DETERMINATION METHODS OF PENTOSIDINE CONCENTRATIONS

It is possible to detect pentosidine at a very low concentration since it is stable under acid hydrolysis conditions of proteins and due to its fluorescent properties. Therefore, this compound may be one of the biomarkers of glycation end-products (AGEs) [17].

From the literature it is known that determination of pentosidine can be performed by: enzyme-linked immunosorbent assay (ELISA) [6, 13-16] and high performance liquid chromatography (HPLC) coupled with different detectors [5, 18-21, 27, 28, 30-31, 38] including tandem mass spectrometry (MS/MS) [33, 34, 36].

According to Scheijen et al. [17] the use of the antisera to immunoassays for AGEs determination is doubtful. These authors claim that reproducibility and sensitivity of these methods are not optimal since the specificity of the antibodies is often difficult to define. Another problem is the fact that not all AGEs epitopes in a protein can interact with the antibodies due to the steric restrictions [17]. Currently, it is possible to purchase commercial immunoassay kits for the determination of pentosidine [14, 15].

According to the authors cited above [17], sensitive method of quantification of pentosidine can be achieved using different chromatographic systems [5, 30]. One such method was developed by Slowik-Zylka et al. [5], where the chromatographic system consisted of Vydac 218TP54 column 250 × 4.6 mm (5 mm particle size) and precolumn Vydac 218GCC 20 × 4.6 mm.

Application of ion exchange chromatography by Saito et al. [35] significantly prolonged the time of analysis, additionally low repeatability of retention times was observed.

Pentosidine was also determined using reversed-phase ion-pair chromatography [28]. This technique was applied by Spacek et al. [28] with the use of a compact CGC glass column Separon SGX C18 (Manufacturer: Tessek Ltd., Prague, Czech Republic), 150 x 3 mm^2 (particles with a diameter of 7 µm).

In turn, in the method proposed by Florid et al. [38] additionally, prior to HPLC analysis (column, Spherisorb ODS2, 5 mm, 15 x 0.4 cm (Waters, Milford, MA, USA) samples were cleaned up using solid phase extraction (SPE) [38].

Scheijen et al. [17] described three main disadvantages of above mentioned methods: long time of analysis, complicated sample preparation steps and the use of expensive equipment LC-MS/MS. Therefore, the authors developed a fast (30 minutes), simple and sensitive RP-HPLC method where column Allsphere ODS-2 (150 mm x 4.6 mm, 3 µm) was applied which enabled to achieve clear chromatograms and high repeatability of retention times.

Slowik-Zylka et al. [5] pointed out that pentosidine, in the plasma samples, is most commonly determined by high performance liquid chromatography. It is usually one-step procedure where reverse phase ion pair chromatography (IP RP-HPLC) is used with octadecylsilane (C18 ODS) columns such as Supelcosil LC-318 column (Supelco), Vydac 218 TP [22], TSK-GEL ODS-80T (Tosoh) [7, 37], Wakosil-II 5C18AR (Wako) [23] or Hi-Pore RP-318 (Bio-Rad) [26]. However, according Slowik-Zylka et al. [5], one-step analysis shows poor specificity, moreover artifacts formed in acid hydrolysis may interfere with pentosidine [5]. To solve this problem, some authors used Sephadex C-25 column [24] or cellulose CF-11 column [23] in order to clean the sample before its separation on C18 column. The second stage of separation, on cation exchanger SP-5PW (Waters) [19], is more useful, but increases significantly the total time of analysis.

Slowik-Zylka et al. developed a sensitive and specific method for the determination of total pen-

tosidine concentration in the plasma using two chromatographic systems with the same C18 column – Vydac 218TP54 – first mobile phase contained trifluoroacetic acid, while the second eluent contained heptafluorobutyric acid, and total time needed to obtain chromatograms was 30 min. Pentosidine was determined using fluorescence detector – excitation wavelength 330 nm and emission 373 nm – the maximum absorbance was observed at wavelength of 325 nm [5].

Pentosidine in urine is mostly determined by HPLC, samples do not require hydrolysis since this compound is present in the free form in urine. Vos et al. [32] analyzed pentosidine in the urine of patients in end stage of osteoarthritis. After dilution (1: 1 in 0.025% H_2SO_4), samples (volume 50 mL) were separated on Whatman partisil 10 SCX (250 x 4.6 mm) column. Samples were eluted using gradient of solvents containing solutions of 0.025% H_2SO_4 and 100 mM $Al_2(SO_4)_3$ in 0.025% H_2SO_4. The column temperature was maintained at 60°C. Pentosidine samples were analyzed using a fluorimeter Jasdo FP-1520 at wavelength of 328 nm (ex) and 378 nm (em) [32].

Sanaka et al. [6] considered the determination of pentosidine by HPLC as time consuming and requiring a complicated procedure of sample pre-treatment, as well as expensive equipment. Therefore, they indicated a need to develop a simple and rapid method of ELISA for pentosidine determination. Izuhara et al. [16] applied this technique to determine the concentration of pentosidine in the plasma of patients with diabetes (mean value of determined biomarker was 0.18 nmol/mL of plasma), patients on hemodialysis (1.52 nmol/mL) as well as in the control group (0.16 nmol/mL). RP-HPLC (C18 column, Waters, Tokyo, Japan) analysis was performed to compare the results. Authors obtained the same mean concentration of pentosidine for both groups of patients, whereas in the control group the result was slightly lower – about 0.02 nmol/mL lower concentration.

Recent scientific reports suggest the possibility of using ELISA kits for determination of pentosidine in biological material. Kerkeni et al. [14] used this technique to determine the concentration of pentosidine in patients with type 2 diabetes. Microplates were pre-coated with an antibody specific for human pentosidine. After addition of the sample, incubation and washing step, the plates were incubated with horseradish peroxidase (HRP), then 3,3',5,5'-tetramethylbenzidine (TMB) was used as a substrate for the reaction and the absorbance was read at the corresponding wavelength [14].

4. PENTOSIDINE LEVELS IN BIOLOGICAL FLUIDS AND HUMAN TISSUES

Pentosidine is determined mainly in blood [17, 18, 25, 29], less often in urine [25, 28, 32, 33, 37], saliva [15], collagen [32], bones, skin, cartilage [28, 35]. It is analyzed, inter alia, in patients with: diabetes [14], osteoarthritis [25, 28, 32], renal failure, dialysis and kidney transplant [13, 14, 17, 18, 33, 36, 37], in diabetic retinopathy [14, 16, 29] and in patients with asthma [15].

This compound can be determined both as free form and bound to protein. The concentrations of pentosidine in the blood of healthy subjects, as described in the literature, vary from 0.21 pmol to 1.4 pmol/mg of protein [5, 17, 19, 29, 38] for a total pentosidine and from 0.25 ng/mL to 3.3 ng/mL [14, 15] or from 0.79 pmol/mL to 151 pmol/mL for the free pentosidine [13, 16, 33, 36]. These differences may result from the type of method applied for pentosidine determination and selection of control groups.

According to various authors, the concentration of pentosidine in the urine of healthy individuals is about 14 pmol/mL [33]; 5.2 nmol/mmol creatinine [37]; or 6.2 nmol/mmol creatinine [28]. Takahashi et al. determined pentosidine concentration in urine samples of patients with diabetes (8.7 nmol/mmol creatinine), osteoporosis (7.9 nmol/mmol creatinine) and patients with chronic renal failure – 36.1 nmol/mmol creatinine [37].

Scheijen et al. [17] marked concentration of pentosidine bound to protein in plasma of patients with end-stage of renal failure. In this group of patients the mean concentration of pentosidine was 3.05 pmol/mg protein, whereas in the control group 0.21 pmol/mg protein.

Other authors, Floridi et al. [38], also conducted similar studies in the group of hemodialysis patients. In this case, the mean concentration of the analyzed biomarker was 27.3 pmol/mg protein (in the control group – 1.4 pmol/mg).

Slowik-Zylka et al. [18] monitored the changes of pentosidine concentration in the plasma of patients after kidney transplantation, and in this case the decrease of the analyzed compound was observed (from 27.7 to 6.48 pmol/mg of protein

at 60 day after transplantation, in the control group the mean concentration of pentosidine was 1.27 pmol/mg). At the same time the authors determined the concentration of free pentosidine and again the reduction in the concentration of analyte was observed (from 22.1 to 1.39 pmol/mL, control group – 0.79 pmol/mL).

Salman et al. [29] studied the pentosidine concentration in the blood in patients with diabetes (mean concentration of 6.9 pmol/mg protein in the study group; in control group – 0.9 pmol/mg). Kerkeni et al. [14] determined concentration of pentosidine in the serum of patients with diabetic nephropathy and retinopathy, and the mean concentration of studied compound was 287.58 and 337.79 pg/mL, respectively (controls – 246.03 pg/mL). The authors of the cited study found that determination of the pentosidine concentration may be a biomarker for monitoring the course of microvascular complications in patients with type 2 diabetes.

Mitsuru Saito et al. [35] analyzed concentration of pentosidine in articular cartilage samples, skin, and diaphysial femur and obtained the values of 0.002; 0.001 and 0.01 mol/mol collagen, respectively.

However, Kanazawa et al. [15] found that the concentrations of pentosidine in induced sputum were significantly higher in asthma patients (mean concentration of 23.9 ng/mL) than in controls (3.3 ng/mL). These authors also observed higher concentrations of pentosidine in older people comparing to younger individuals, and in smokers than in non-smokers (young non-smoker – 1.9 ng/mL, elderly non-smoking individuals – 9.6 ng/mL, and young smokers – 5.3 ng/mL).

5. SUMMARY

The problem of the choice of method for the determination of pentosidine has been discussed for many years. The use of a specific analytical technique depends on many factors such as: the type of material for analysis, sample preparation, analysis of costs and the time required for analysis performance. Aiming for the use of the sample minimal volume and reduction of the use of toxic solvents, as well as cost and time of analysis, also requires the need of high specificity of method of pentosidine determination in biological material. Chromatographic methods dominate and outperform the immunochemical technique in this respect, especially when they involve the use of tandem mass spectrometers.

Developing a standardized and validated procedures for determination of pentosidine in the specific biological material as well as for the specific disease could provide the basis for a reliable database. Future use of pentosidine as a sensitive and specific biomarker of the exposure to compounds of advanced stages of Maillard reaction (especially these ones from the group of AGEs) entails the need for such data based on standardized analytical procedure.

REFERENCES

[1] Warwas M., Piwowar A., Kopiec G., Zaawansowane produkty glikacji (AGE) w organizmie – powstawanie, losy, interakcja z receptorami i jej następstwa, Farm. Pol., 66, 2010, 8, 585-590.

[2] Michalska A., Zieliński H., Produkty reakcji Maillarda w żywności, ŻNTJ, 2, 2007, 51, 5-16.

[3] Jabłońska-Trypuć A., Czerpak R., Rola nieenzymatycznej glikozylacji białek w procesach starzenia organizmu i patogenezie chorób wieku podeszłego, Post. Biol. Komórki, 34, 2007, 4, 683-693.

[4] Schlaffke J., Ocena zawartości zaawansowanych produktów glikacji białek w skórze metodą autofluorescencji u chorych na cukrzycę typu 1, Rozprawa na stopień doktora nauk medycznych, Poznań 2013.

[5] Slowik-Zylka D., Safranow K., Dziedziejko V., Bukowska H., Ciechanowski K., Chlubek D., A sensitive and specific HPLC method for the determination of total pentosidine concentration in plasma, J. Biochem. Bioph. Methods, 61, 2004, 313-329.

[6] Sanaka T., Funaki T., Tanaka T., Hoshi S., Niwayama J., Taitoh T., Nishimura H., Higuchi C., Plasma pentosidine levels measured by a newly developed method using ELISA in patients with chronic renal failure, Nephron, 91, 2002, 64-73.

[7] Hashimoto H., Arai K., Chikuda M., Obara Y., Relationship between pentosidine and pyridinoline levels in human diabetic cataract lenses, J. Clin. Biochem. Nutr., 47, 2010, 233-237.
[8] Śliwińska A., Łysiak-Szydłowska W., Metody oznaczania końcowych produktów glikacji białek – AGEs, Diagn. Lab., 41, 2005, 85-94.
[9] Goldin A., Beckman J. A., Schmidt A. M., Creager M. A., Advanced glycation end products sparking the development of diabetic vascular injury, Circulation, 114, 2006, 597-605.
[10] Takeuchi M., Takino J., Yamagishi S., Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: a novel therapeutic strategy, Curr. Drug Targets, 11, 2010, 11, 1468-1482.
[11] Poulsen M. W., Hedegaard R. V., Andersen J. M., de Courten B., Bügel S., Nielsen J., Skibsted L. H., Dragsted L. O., Advanced glycation end products in food and their effects on health, Food Chem. Toxicol., 60, 2013, 10-37.
[12] Sell D., Monnier V., Structure elucidation of a senescent crosslink from human extracellular matrix, J. Biol. Chem., 264, 1989, 21597-21602.
[13] Taneda S., Monnier V. M., ELISA of pentosidine, an advanced glycation end product, in biological specimens, Clin. Chem., 40, 1994, 1766-1773.
[14] Kerkeni M., Saïdi A., Bouzidi H., Letaief A., Yahia S. B., Hammami M., Pentosidine as a biomarker for microvascular complications in type 2 diabetic patients, Diabetes Vasc. Dis. Res., 10, 2012, 3, 239-245.
[15] Kanazawa H., Kyoh S., Tochino Y., Asai K., Hirata K., Potential role of pentosidine on susceptibility to small airway closure in elderly and smoking asthma, Resp. Med., 108, 2014, 709-715.
[16] Izuhara Y., Miyata T., Ueda Y., Suzuki D., Asahi K., Inagi R., Sakai H., Kurokawa K., Sensitive and specific ELISA for plasma pentosidine, Nephrol. Dial. Transpl., 14, 1999, 576-580.
[17] Scheijen J. L. J. M., van de Waarenburg M. P. H., Stehouwer C. D. A., Schalkwijk C. G., Measurement of pentosidine in human plasma protein by a single-column high-performance liquid chromatography method with fluorescence detection, J. Chromatogr. B, 877, 2009, 610-614.
[18] Slowik-Zylka D., Safranow K., Dziedziejko V., Ciechanowski K., Chlubek D., Association of plasma pentosidine concentrations with renal function in kidney graft recipients., Clin. Transplant., 24, 2010, 839-847.
[19] Odetti P., Fogarty J., Sell D., Monnier V., Chromatographic quantitation of plasma and erythrocyte pentosidine in diabetic and uremic subjects, Diabetes, 41, 1992, 153-159.
[20] Viguet-Carrin S., Gineyts E., Bertholon C., Delmas P. D., Simple and sensitive method for quantification of fluorescent enzymatic mature and senescent crosslinks of collagen in bone hydrolysate using single-column high performance liquid chromatography, J. Chromatogr. B, 877, 2009, 1-7.
[21] Mikulíková K., Eckhardt A., Kuneš J., Zicha J., Mikšík I., Advanced glycation end-product pentosidine accumulates in various tissues of rats with high fructose intake, Physiol. Res., 57, 2008, 89-94.
[22] Grandhee K., Monnier V. M., Mechanism of formation of the Maillard protein cross-link pentosidine, J. Biol. Chem., 266, 1991, 11649-11653.
[23] Yoshihara K., Nakamura K., Kanai M., Nagayama Y., Takahashi S., Saito N., et al., Determination of urinary and serum pentosidine and its application to elder patients, Biol. Pharm. Bull., 21, 1998, 1005-1008.
[24] Takahashi M., Kushida K., Kawana K., Ishihara C., Deneda M., Inoue T., Horiuchi K., Quantification of the cross-link pentosidine in serum from normal and uremic subjects, Clin. Chem., 39, 1993, 10, 2162-2165.
[25] Braun M., Hulejová H., Gatterová J., Filková M., Pavelková A., Sléglová O., Kaspríková N., Vencovsky J., Pavelka K., Senolt L., Pentosidine, an advanced glycation end-product, may reflect clinical and morphological features of hand osteoarthritis, TORJ, 6, 2012, 64-69.
[26] Dyer D., Blackledge J. A., Thorpe S., Baynes J., Formation of pentosidine during nonenzymatic browning of proteins by glucose, J. Biol. Chem., 266, 1991, 11654-11660.
[27] Ahmed N., Argirov O. K., Minhas H. S., Cordeiro C. A. A., Thornalley P. J., Assay of advanced glycation endproducts (AGEs): surveying AGEs by chromatographic assay with derivatization by

6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate and application to N_{ε}-carboxymethyl-lysine and N_{ε}-(1-carboxyethyl)lysine-modified albumin, Biochem. J., 364, 2002, 1-14.
[28] Spacek P., Adam M., Pentosidine in osteoarthritis: HPLC determination in body fluids and in tissues, Rheumatol. Int., 26, 2006, 923-927.
[29] Salman A. G., Mansour D. E. A. A., Swelem A. A., Al-Zawahary W. M. A., Radwan A. A., Pentosidine – a new biochemical marker in diabetic retinopathy, Ophthalmic Res., 42, 2009, 96-98.
[30] Wilker S. C., Chellan P., Arnold B. M., Nagaraj R. H., Chromatographic quantification of argpyrimidine, a methylglyoxal-derived product in tissue proteins: comparison with pentosidine, Anal. Biochem., 290, 2001, 353-358.
[31] Chen J. R., Takahashi M., Kushida K., Suzuki M., Suzuki K., Horiuchi K., and Nagano A., Direct detection of crosslinks of collagen and elastin in the hydrolysates of human yellow ligament using single column high performance liquid chromatography, Anal. Biochem., 278, 2000, 99-105.
[32] Vos P. A. J. M., Mastbergen S. C., Huisman A. M., de Boer T. N., DeGroot J., Polak A. A., Lafeber F. P. J. G., In end stage osteoarthritis, cartilage tissue pentosidine levels are inversely related to parameters of cartilage damage, Osteoarthr. Cartil., 20, 2012, 233-240.
[33] Thornalley P. J., Battah S., Ahmed N., Karachalias N., Agalou S., Babaei-Jadidi R., Dawnay A., Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry, Biochem. J., 375, 2003, 581-592.
[34] Babaei-Jadidi R., Karachalias N., Ahmed N., Battah S., Thornalley P. J., Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine, Diabetes, 52, 2003, 2110-2120.
[35] Saito M., Marumo K., Fujii K., Ishioka N., Single-column high-performance liquid chromatographic – fluorescence detection of immature, mature, and senescent cross-links of collagen, Anal. Biochem., 253, 1997, 26-32.
[36] Agalou S., Ahmed N., Dawnay A., Thornalley P. J., Removal of advanced glycation end products in clinical renal failure by peritoneal dialysis and haemodialysis, Biochem. Soc. T., 31, 2003, 6, 1394-1396.
[37] Takahashi M., Hoshino H., Kushida K., Kawana K., Inoue T., Direct quantification of pentosidine in urine and serum by HPLC with column switching, Clin. Chem., 42, 1996, 1439-1444.
[38] Floridi A., Trizza V., Paolotti P., Lucarelli C., Analytical strategy for the assessment of the protein glycation status in uremic patients by high-performance liquid chromatography, J. Chromatogr. A, 846, 1999, 65-71.