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Large volume sample stacking for rapid and sensitive determination of antidiabetic drug metformin in human urine and serum by capillary electrophoresis with contactless conductivity detection

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I would like dedicate this paper to my teacher Prof. František Opekar.

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ABSTRACT

Two CE methods with contactless conductivity detection have been developed for determining the oral antidiabetic drug metformin in human urine and blood. The determination of metformin is performed on a separation capillary with an effective length of 14 cm, using a maximum voltage of 30 kV and with a small injection of 50-fold diluted urine into the capillary. Under these conditions, the migration time of metformin is 35 s and the LOD is 0.3 μM. Large-volume sample stacking was used to determine low metformin levels in serum. The injection of a sample of serum deproteinized with acetonitrile was 10 times greater compared to the injected amount of urine. This enabled reduction of the LOD to 0.03 μM and the metformin migration time equalled 86 s. The undesirable solvent from sample zone was forced out of the capillary to ensure rapidity and good repeatability of the determination. The RSD values for the migration time are 0.1% for urine and 0.7% for serum; RSD for the peak areas equalled 1.4% for urine and 2.6% for serum. The developed CE technique was tested on performance of routine analyses of metformin in the urine and serum of patients suffering from type II diabetes mellitus.

1. Introduction

Metformin (Fig. 1) is an oral antidiabetic drug of the biguanide group. At the present time, metformin is the drug of choice for treating type II diabetes and is prescribed primarily for overweight people (www.idf.org). Clinical studies have demonstrated that ten-year administration of metformin to obese diabetics reduces morbidity and mortality by 30% compared to treatment with insulin and sulphonyl urea [1]. In addition, administration of metformin to obese and overweight diabetics does not contribute to a further increase in their weight [2]. Metformin is further characterized by a low risk of lactic acidosis compared to other biguanides [3]. At the present time, metformin is the most extensively used antidiabetic drug and 48 million packages were prescribed in 2010 in the U.S.A. alone [4]. In addition to treatment of advanced type II diabetes, metformin is also used to treat gestational diabetes [5] and prediabetes in high-risk groups. Metformin is also used to treat polycystic ovary syndrome [6] and successful use in cancer prevention has also been described [7].

The plant Galega officinalis [8] is a natural source of drugs in the biguanide group. However, metformin is produced synthetically for use in contemporary medicine [9]. A large number of analytical methods have been developed for controlling the purity of drugs, determining metformin in plant products and in clinical samples: HPLC with MS [10–13] or UV detection [14–17]; hollow fibre extraction with consequent HPLC determination [18,19]; high-performance thin-layer chromatography [20]; H–NMR in combination with HPLC–MS [21]; GC–MS [22] and direct chemiluminescence determination [23]. The capillary electrophoresis method can be successfully used to determine ionogenic metformin, which is also highly soluble in water. In the analysis of biological and clinical samples, CE is characterized by low requirements on pretreatment of a complicated sample matrix compared to HPLC and GC. Only a few references in the literature describe the CE determination of metformin in pharmaceutical products [24–28], blood plasma [29–32] and urine [33].

This work is concerned with the development of a very rapid CE method for determination of metformin in human urine and blood [34]. Treatment of urine and serum for CE analysis is very simple and consists only in diluting the body fluid and, as appropriate,

Abbreviations: ACN, acetonitrile; BGE, background electrolyte; C^4D, capacitively coupled contactless conductivity detection; ECE, electrochemiluminescence; EOF, electroosmotic flow; FASS, field amplified sample stacking; HAc, acetic acid; INST, coating solution for fused silica capillary; LOD, limit of detection; LOQ, limit of quantification.

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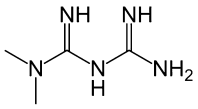

Fig. 1. Structure of metformin (N,N-dimethylimidodicarbonimidic diamide).

deproteinization of the sample. The high sensitivity of the determination when using universal capacitively coupled contactless detection (C^4D) [35–39] is based on separation in a short capillary, where excessive broadening of the sample is eliminated, and also on on-line preconcentration of the sample using large-volume sample stacking [40–44].

2. Experimental

2.1. CE experiments and capillary arrangement

Electrophoretic measurements were carried out using the $HP^{3D}CE$ system (Agilent Technologies, Waldbronn Germany) equipped with a C^4D detector [45], which is placed in the electrophoretic cassette thermostated at constant temperature of 25 °C.

All CE separations of urine and serum samples were performed in a fused-silica capillary (Composite Metal Services, UK), 50 μm I.D., 363 μm O.D., 31.7 cm total length. This is the minimum capillary length for $HP^{3D}CE$ systems. The inner surface of the capillary was covered using INST coating solution (Biotaq, U.S.A.) to prevent electro-osmotic flow (EOF) [46]. A new capillary was washed stepwise with 0.1 M NaOH (5 min), water (5 min), INST coating solution (2 min), water (5 min) and BGE (5 min). 0.5 min washing with the BGE was used between individual analyses. All the separations were carried out in the optimized background electrolyte (BGE) of 2.0 M acetic acid (pH 2.15).

The C^4D detector is placed in cassette asymmetrically to the electrophoretic capillary, 17.0 cm from the one end (called the long end) and 14.7 cm from the other end (called the short end). Two different sets of experimental conditions were used for determination of metformin in urine (i) and serum samples (ii):

i) Urine samples, treated by dilution with water, were injected into the short end of capillary in a small amount (pressure 50 mbar for 2 s, corresponding to a sample zone length of 2.3 mm and a sample zone volume of 4.5 nL). Then the maximal voltage of +30 kV (in relation to the short end of capillary, current 36.8 μA) was switched on and simultaneously the sample zone was forced out of the capillary by application of negative pressure. The hydrodynamic impulse used to force the sample out of the capillary was the same as the injection impulse, 100 mbar s. After turning on the separation voltage, the metformin cations and other ions rapidly leave the sample zone and subsequently the remaining solvent in the sample zone is forced out of the capillary. When the sample zone was not forced out of the capillary, the electric current was interrupted during the separation. The explanation is, that after the ions have left, the sample zone presents a large resistance for passage of the electric current during the separation.
ii) Serum samples, deproteinized by addition of acetonitrile, were injected into the long end of the capillary in a large volume (pressure 50 mbar for 20 s, corresponding to a sample zone with a length of 22.9 mm and a volume of 45 nL). Then a separation voltage of +20 kV was turned on (current 21.5 μA) and simultaneously the acetonitrile zone was forced out of the capillary by application of a negative pressure of −50 mbar for 20 s. Metformin again leaves the acetonitrile zone and enters the surrounding BGE which, if the acetonitrile zone were not removed, would constitute an obstacle for passage of the electric current.

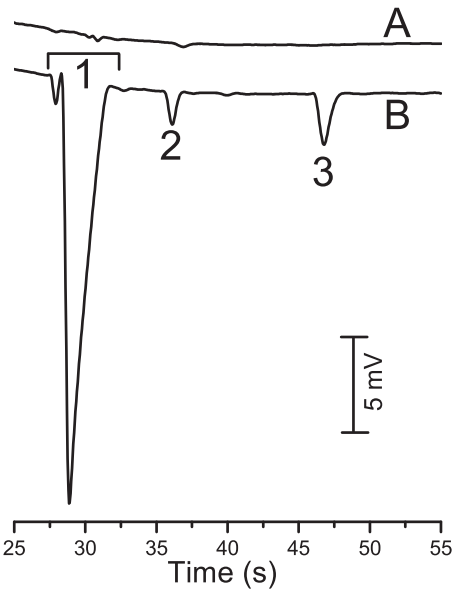

Fig. 2. Electropherogram of diabetic urine diluted 50-fold with BGE (A) and diluted 50-fold with water (B). Peak identification: inorganic cations (1), metformin (2), creatinine (3).

2.2. Chemicals

All the chemicals used were of *p.a.* purity: acetic acid (HAc, Sigma), creatinine (Fluka), metformin hydrochloride (Fluka), NaOH (Fluka), HCl (Sigma), acetonitrile (ACN, Fluka). The stock solutions of metformin and creatinine were prepared at concentrations of 10 mM. Deionized Milli-Q water (18.2 MΩ cm, Millipore, Molsheim, France) was used for preparation of the BGE and the stock solutions of the standards, which were stored in a refrigerator at 4 °C.

2.3. Pretreatment of urine and serum samples

Samples of morning urine and venous blood were collected from patients suffering from type II diabetes mellitus, undergoing treatment at the 2nd Department of Internal Medicine, Královské Vinohrady Faculty Hospital in Prague. Metformini hydrochloridum was administered to them in an amount of 1000 mg per day. The control urine and blood samples were obtained from healthy adult volunteers.

The collected urine samples were stored in a freezer at −20 °C and maintained at this temperature until the analysis. Prior to the CE measurement, unfrozen urine samples were only diluted 50-fold with 0.01 M HCl acid (20 μL of urine were added to 980 μL of 0.01 M HCl). Dilution of the urine with acidified water is very important for sharpening the sample zone; water suppresses the conductivity of the sample and causes it to become shorter. Acidification of the sample with HCl maintains the metformin in the protonated form, which rapidly leaves the sample zone. The importance of water for sharpening the sample zone is clearly demonstrated in Fig. 2. Simultaneously, Fig. 2 depicts a recording of 50-fold dilution of the urine with the BGE, which does not lead to sample sharpening and the peaks of the analyte are lost in the detector noise.

Serum samples obtained from coagulated venous blood were stored in a freezer at −20 °C until the analysis. Before analysis, the unfrozen serum samples were deproteinized by mixing 100 μL of serum with 300 μL of acetonitrile acidified with HCl at a concentration of 0.01 M. Deproteinization was performed in an Eppendorf tube after 30 s shaking. Then the serum samples were filtered through a Durapore polyvinyl difluoride membrane (pore size 0.45 μm, centrifugal filter devices, Millipore, Bedford, USA) and