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Novel MEKC method for determination of sodium 2-mercaptoethanesulfonate in human plasma with in-capillary derivatization and UV detection

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ABSTRACT

Sensitive electrophoretic method for determination of total sodium 2-mercaptoethanesulfonate (mesna) in human plasma, based on the stacking with high salt concentration in MEKC and in-capillary derivatization with 2-chloro-1-methyllepidinium tetrafluoroborate followed by UV detection was developed. In the method 0.03 mol L^{-1} pH 7 phosphate buffer with the addition of 0.01 mol L^{-1} SDS, and 10% ACN was used as a BGE. The limit of quantification (LOQ) of the method was 0.5 μmol L^{-1}. Linearity in detector response was observed over the range of 0.5–10 μmol L^{-1} with the correlation coefficient 0.9971. The intra- and inter-day accuracy (three concentration levels, 5 days, n = 3) of the method ranged from 97.2 to 110.0% and from 94.0 to 101.2%, respectively. The novel MEKC method with UV detection proved to be suitable for determination of total mesna in human plasma.

1. Introduction

Sodium 2-mercaptoethanesulfonate (mesna) belongs to the group of thiol compounds used in some cancer therapy to prevent side-effects of oxazaphosphorine, cyclophosphamide and ifosfamide. In urine free thiol group of mesna reacts with ifosfamide and cyclophosphamide, responsible for the toxic effect on bladder, affording harmless to the mucous membrane derivatives [1–4]. Ifosfamide, an oxazaphosphorine alkylating agent is concurrently administered with the uroprotective mesna, which prevents the occurrence of hemorrhagic cystitis. Formed in the blood by the autoxidation inactive disulfide of mesna – dimesna can be back converted to reduced active form in the renal tubule cells, thereby inactivating toxins like acrolein to the bladder. Moreover, mesna acts as mucolytic drug that efficiently cleaves disulfide bonds of mucus polypeptides [2–6]. Due to the small molecular size, mesna is prone to concentrate especially in kidneys [1,7]. To the best of our knowledge there is no data supporting statement concerning the toxic effect of mesna accumulated in kidneys. Contrary, it has been proved, that accumulation of mesna in the kidney could help in minimizing renal ischemic reperfusion injury [8]. Because of the biological importance of mesna, significant efforts have been made to develop a robust and reliable methods for its determination in plasma and urine.

High performance liquid chromatography (HPLC) is already an established technique for quantification of thiol compounds in different biological matrices. For the determination of mesna, both in pharmaceuticals and biological samples, HPLC with electrochemical [7,9,10], UV [11–13] and fluorescence [8,14] detection as well as flow injection technique [15,16] were used. While liquid chromatography based methods are sensitive and precise, they produce a large amounts of toxic wastes. Thus, reducing or eliminating the use of hazardous solvents is therefore an important goal in terms of environmental conservation, human health and the economy.

To the best of our knowledge there are no capillary electrophoresis (CE) methods for the determination of mesna in biological fluids. It is commonly known that main limitation of CE techniques applies to low concentration sensitivity which prevents the detection of trace levels of analytes. Low sensitivity arises from two sources, namely small sample volumes and short optical pathlengths. To overcome this problem various in-line preconcentration

Abbreviations: CMLT, 2-chloro-L-methyllepidinium tetrafluoroborate; Mesna, Sodium 2-mercaptoethanesulfonate; MPS, Sodium 3-mercaptopropanesulfonate; TCEP, Tris(2-carboxyethyl)phosphine.

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techniques have been developed and well described over the past years [17]. One of them, so-called stacking enables preconcentration of neutral and ionic analytes. On-line stacking allows to determine very low concentration of analytes in biological matrices while high sensitivity and selectivity of method as well as efficient distribution of charged molecules puts it as one of the most interesting modern analytical techniques [18–21].

It is also commonly known that a large number of steps during sample preparation increase the likelihood of incorporating error into the final measurement. In general, any tool helps to reduce the number of experiments shortens the time, cost as well as minimizes total error of analytical method.

Methodology described in this paper opens up the possibility of analyzing plasma for total mesna using MEKC technique with UV detection. In our approach the derivatization reaction of mesna is carried out inside capillary during the electrophoretic separation, thus sample handling affecting total error and sample preparation time is significantly reduced.

2. Experimental

2.1. Reagents

Tris(2-carboxyethyl)phosphine (TCEP), 2-mercaptoethanesulfonic acid, sodium salt (mesna), trimethyloxonium tetrafluoroborate and 3-mercaptopropanesulfonic acid, sodium salt (MPS) used as internal standard were received from Fluka (Buchs, Switzerland). Sodium dodecyl sulfate (SDS) was from Sigma (St. Louis, MO, USA). 2-Chloro-1-methyllepidinium tetrafluoroborate (CMLT) was synthesized in this laboratory according to the procedure described earlier [22]. Disodium hydrogen phosphate heptahydrate ($Na_2HPO_4{\cdot}7H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), phosphoric acid (H_3PO_4), acetonitrile (ACN) and sodium hydroxide (NaOH) were purchased from J.T. Baker (Deventer, The Netherlands). Deionized water was used for the preparation of all the solutions. Water was purified using a Millipore Milli-Q-RG System (Watford, U.K.).

2.2. Solutions

Standard solutions of mesna and MPS (100 mmol L^{-1}) were prepared in deionized water and stored at 4 °C. Stock solutions of 0.25 mol L^{-1} TCEP and 0.1 mol L^{-1} CMLT were prepared in 1 mL of 0.2 mol L^{-1} phosphate buffer, pH 7.8. The pH of buffers was adjusted by potentiometric measurements. The pH-meter was calibrated with standard pH solutions. In the case of pH 7 phosphate buffer 0.03 mol L^{-1} disodium hydrogen phosphate heptahydrate was titrated with 0.03 mol L^{-1} sodium dihydrogen phosphate dihydrate. Background electrolyte containing SDS and ACN was also prepared immediately before use.

2.3. Instrumentation and CE conditions

A Hewlett-Packard 3D Capillary Electrophoresis System (Waldbronn, Germany) equipped with a UV–vis detector set at λ=346 nm and automatic injector was used. Uncoated fused silica capillary (Polymicro Technologies, Phoenix, USA) with an effective length of 41.5 cm and a total length of 50 cm (75 μm id) was served as the separation column. The temperature of the capillary was controlled by the air thermostatic system and maintained at 25 °C. The ChemStation software to control CE instrument, data acquisition, peaks area and migration times measurements was applied. Before each MEKC analysis the capillary was washed (pressure about 1 bar) with deionized water (5 min) and 0.1 mol L^{-1} NaOH (5 min), then deionized water (5 min) and finally phosphate buffer (0.03 mol L^{-1}, pH 7) with the addition of SDS (0.01 mol L^{-1}) and 10% of ACN (30 min). The analyses were conducted at a voltage of 28 kV.

2.4. Calibration standards

Working solutions of mesna and MPS were prepared by dilution of standard solutions in deionized water as needed. In order to prepare the calibration standards of total mesna, 50 μL of plasma from apparently healthy volunteer donor was spiked with increasing amount of the working mesna solution to obtain final concentrations of 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7.5, 10 μmol L^{-1} and fixed quantity of MPS (4.76 μmol L^{-1}). The calibration standards (in three replicates) were prepared according to the procedure described in Section 2.5.

2.5. Samples and calibration standards preparation

Plasma samples donated by apparently healthy volunteers were examined during experiments. Some plasma samples from volunteers after oral administration of 500 mg mesna were also analyzed. The blood was taken after 2.5 h and plasma was immediately prepared. To 250 μL of plasma 5 μL of 0.25 mol L^{-1} TCEP was added in order to reduce of disulfide bonds. After 10 min samples were centrifuged on cut-off filters (10 kDa, Sartorius, Vivaspin 500, polyethersulfone membrane) for 15 min (15000*g*) to remove proteins and filtrate was assayed immediately or stored at −80° C. Directly before CE analysis, to 50 μL of deproteinized plasma 50 μL of phosphate buffer (0.2 mol L^{-1}, pH 7.8) and 5 μL of 0.1 mmol L^{-1} MPS (internal standard) were added. In case of preparation of calibration standards or precision and accuracy standards to a sample also mesna was added. Finally an aliquot was injected into electrophoretic system.

The investigation was performed after approval by the Ethical Committee of the University of Łódź.

2.6. In-capillary sample derivatization

At the beginning optimization of in-capillary derivatization conditions was performed. Different parameters such as time and pressure of sample and CMLT injection, concentration and pH of derivatization buffer, time and voltage of mixing zones were checked. Optimization of the in-capillary derivatization of mesna and MPS was carried out by CZE. In this section final in-capillary derivatization MEKC analysis settings are presented. Developed procedure for determination of mesna in human plasma was consisted of several steps. First, the capillary was conditioned with BGE containing SDS and ACN. The derivatization of mesna and MPS inside capillary was carried out by consecutive introduction of plasma sample (5 s, 30 mbar), 0.2 mol L^{-1}, pH 7.8 phosphate buffer (3 s, 30 mbar) and 50 mmol L^{-1} CMLT (0.8 s, 30 mbar). Then, for 96 s the voltage of 9 kV to enable mixing of zones was applied and next separation voltage of 28 kV was used.

3. Results and discussion

In this work, we put interesting data about the novel electrophoretic method for determination of mesna in human plasma using micellar electrokinetic chromatography and UV detection. Unfortunately, similarly to other biologically important thiols, mesna does not markedly absorb radiation in UV region. Moreover, mesna exhibits high susceptibility to oxidation forming disulfide dimers with other thiol compounds present in plasma. In order to overcome these issues and make analytes compatible with UV–vis detector as well as to block the labile −SH function, in-capillary derivatization reaction with CMLT was successfully implemented.