Journal of Chromatography A, 1468 (2016) 236–240

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Rapid determination of meldonium in urine samples by capillary electrophoresis with capacitively coupled contactless conductivity detection

Andrea Šlampová, Pavel Kubáň*

*Institute of Analytical Chemistry of the Czech Academy of Sciences, v. v. i., Veveří 97, CZ-60200 Brno, Czech Republic*

ARTICLE INFO

*Article history:*
Received 8 August 2016
Received in revised form 9 September 2016
Accepted 13 September 2016
Available online 13 September 2016

*Keywords:*
Capillary electrophoresis
Contactless conductivity detection
Meldonium
Mildronate
Urine

ABSTRACT

Capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-$C^4D$) was employed for fast determination of meldonium (MEL) in urine samples. Background electrolyte consisting of 2 M acetic acid (pH 2.3) was used for separation of MEL from cationic compounds present in urine samples and the overall analysis time was less than 4 min per sample. Direct injection of urine samples was possible after 1:9 dilution with deionized water. This simple sample pretreatment was sufficient to eliminate possible matrix effects on CE performance and allowed for precise and sensitive determination of free MEL in urine. Excellent linearity ($r^2 \geq 0.9998$) was obtained for two concentration ranges, 0.02–4 μg mL$^{-1}$ and 2–200 μg mL$^{-1}$, by simply changing injection time from 10 to 2 s without the need for additional dilution of urine samples. Limit of detection was 0.015 μg mL$^{-1}$ and average recoveries from urine samples spiked at 0.02–123.5 μg mL$^{-1}$ MEL ranged from 97.6–99.9%. Repeatability of migration times and peak areas was better than 0.35% and 4.2% for intraday and 0.95% and 4.7% for interday measurements, respectively. The above reported data proved good applicability of the CE-$C^4D$ method to determination of various MEL concentrations in urine samples and good long-term performance of the analytical system. The method might be particularly useful in analyses of large batches of samples for initial testing of MEL-positive vs. MEL-negative urine samples.



## 1. Introduction

Meldonium (MEL), trade-named also as mildronate, is a limited-market pharmaceutical developed in 1970s at Latvian Institute of Organic Synthesis. The chemical name of meldonium is 3-(2,2,2-trimethylhydrazinium) propionate dihydrate and is an analogue of carnitine. MEL is a fatty acid oxidation inhibitor and, today, it is principally used to treat coronary artery diseases, angina and heart attacks (myocardial infarction). Its therapeutic activity is gained by altering pathways for carnitine, a nutrient involved in fat metabolism.

Since January 1, 2016, MEL has been added to the World Anti-Doping Agency (WADA) list of banned substances because of "evidence of its use by athletes with the intention of enhancing performance" [1,2], and has been classified by WADA as a metabolic modulator. MEL demonstrates an increase in endurance performance of athletes, improved rehabilitation after exercise, protection against stress, and enhanced activation of central nervous system functions [3]. On April 13, 2016, WADA has issued updated guidelines allowing less than 1 μg mL$^{-1}$ of MEL for samples collected before March 1, 2016 due to the long half-time life of the drug in human body [4].

MEL is a hydrazinium base of low molecular weight with a simple structure (see Fig. 1). Analytical determination of MEL is, however, complicated due to the lack of UV-absorbing chromophores in its structure and MEL is thus not suitable for analytical techniques with conventional UV–vis absorbance detection. Despite this, different assays were developed for the determination of MEL in pharmaceutical formulations, human plasma and urine. MEL was determined by high performance liquid chromatography (HPLC) with direct UV-absorbance detection (after derivatization) [5] and evaporative light scattering detection [6,7] or by voltammetry [8]. Micellar electrokinetic chromatography coupled with mass spectrometry was used for studies of MEL stability in aqueous solutions [9]. Nevertheless, it has been reported that HPLC with mass spectrometry (HPLC–MS) or tandem mass spectrometry (HPLC–MS/MS) are the most selective and sensitive methods for the determination of MEL [3,10–13].

HPLC–MS/MS is, however, a costly method and usually requires time-consuming sample pretreatment when body fluids are ana-

* Corresponding author.
*E-mail address:* kuban@iach.cz (P. Kubáň).

http://dx.doi.org/10.1016/j.chroma.2016.09.027
0021-9673/

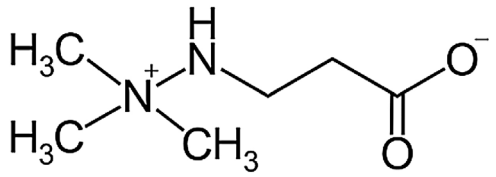

**Fig. 1.** Chemical structure of meldonium (MEL).

lyzed. MEL was determined in plasma and urine samples after precipitation with organic solvent, evaporation and reconstitution [11], after precipitation with organic solvent and centrifugation [12] or alternatively after dilution and centrifugation [11]. Recently, it has been also shown that MEL can be determined in urine samples after precipitation with acetonitrile or after dilution only [3]. Limits of detection of the HPLC–MS/MS assays were usually in units to tens ng mL$^{-1}$ and separation process took between 5 and 50 min [3,10–12]. Even faster ultra-performance LC–MS/MS determination of MEL was reported for ultra-short hydrophilic interaction liquid chromatography (HILIC) column, however at the expense of reduced separation efficiency [13]. HILIC with high resolution mass spectrometry (HILIC-HRMS) was recently also applied to monitor possible misuse of MEL by athletes [3] and to investigate MEL presence in body fluids after administration of single/multiple MEL doses [14]. Alarming findings were reported for analyses of doping control urine samples with more than 2% positive samples, with minimum and maximum MEL concentrations ranging between 0.1 and 1428 μg mL$^{-1}$ and mean MEL concentrations of 121 and 136 μg mL$^{-1}$ for female and male athletes, respectively [3]. Moreover, slow excretion of MEL was confirmed for administration of multiple doses, reporting MEL concentrations between 1 and 9 μg mL$^{-1}$ in urine samples collected at 4–33 days after administration of the final dose [14].

Many pharmaceuticals and clinically important species are small ions or molecules, which are charged in certain pH ranges, and whereas their determination by standard chromatographic methods might be complicated, they are amenable to separations by capillary electrophoresis (CE). CE often replaces chromatographic techniques in analysis of small ions, particularly due to its favorable characteristics, such as low running costs, minimum sample/reagents consumption, short separation times and excellent separation efficiency. Moreover, as the analytes migrate in the separation medium in form of charged species, presence of UV-absorbing chromophores in their structure is not necessary and they can be directly monitored by means of conductivity detection. Numerous publications on CE with capacitively coupled contactless conductivity detection (CE-C$^4$D) were reported for analyses of pharmaceutically and clinically important compounds recently [15–20]. Fundamental information on CE-C$^4$D and its applications in various fields of analytical chemistry can be additionally found in the following up-to-date review articles [21–27].

In this manuscript, a simple, rapid and cheap analytical method for determination of MEL in urine samples was developed. Sample pretreatment was minimized to dilution of urine samples with DI water and MEL was determined by CE-C$^4$D in ng mL$^{-1}$ to mg mL$^{-1}$ concentration range with a sample-to-sample analysis time of less than 4 min. The developed CE-C$^4$D method should be considered rather complementary than competitive to HPLC–MS/MS since it shows lower sensitivity and selectivity, nevertheless, it ensures rapid determination of clinically relevant concentrations of MEL in urine at a fraction of costs of the hyphenated analytical methods. Minute volumes of samples, minimum sample treatment, high analytical throughput and low cost are the major advantages of the developed method and might be especially beneficial in analyses of large batches of urine samples.

## 2. Materials and methods

### 2.1. Reagents, standard solutions and samples

All chemicals were of reagent grade and deionized (DI) water with resistivity higher than 18 MΩ·cm was used throughout. Stock solutions of meldonium, histidine, arginine, glycine and creatinine (1 mg mL$^{-1}$) were prepared in DI water from meldonium dihydrate (MEL), L-histidine (His), L-arginine (Arg), L-glycine (Gly) and creatinine (Crea); all chemicals were supplied by Sigma, Steinheim, Germany. Background electrolyte (BGE) solutions for CE-C$^4$D were prepared from concentrated acetic acid (Fluka, Buchs, Switzerland) and had final concentrations between 0.1 and 5 M. Optimum BGE solution consisted of 2 M acetic acid and was freshly prepared every week. All solutions were stored at the temperature of 4 °C. Drug-free human urine samples were collected from healthy volunteers at the Institute of Analytical Chemistry; a written informed consent was signed by the volunteers before the experiments. All urine samples were stored at 4 °C, allowed to warm up to the ambient temperature and diluted 1:9 with DI water prior to CE-C$^4$D. For non-spiked urine samples, 5 μL of urine and 45 μL of DI water was pipetted into a CE microvial (Agilent Technologies, Waldbronn, Germany, P/N 9301-0978). For spiked urine samples, 5 μL of urine was pipetted into the CE microvial, spiked with appropriate amount of MEL and filled to 50 μL with DI water. The diluted urine samples were vortexed for 30 s and used for direct injection into a separation capillary without any further treatment. For calibration purposes, urine sample spiked with highest MEL concentration was prepared according to the above procedure and other calibration solutions were prepared by dilutions of this sample with 1:9 diluted urine.

### 2.2. Instrumentation

7100 CE instrument (Agilent Technologies) equipped with a C$^4$D (Admet, Prague, Czech Republic, operated at 50 $V_{pp}$ and 1.84 MHz) was used for CE measurements. Fused-silica separation capillaries (50/375 and 25/375 μm i.d./o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA) and were cut to desired lengths. Total lengths ($L_{tot}$) and effective lengths ($L_{eff}$) were 55 or 65 cm and 42 or 52 cm, respectively. Specifications on capillary lengths can be found in corresponding texts and Figure captions. Before use, new capillary was sequentially flushed at 950 mbar for 10 min with 1 M NaOH, DI water and BGE solution. Capillary flushing between two CE runs was optimized to achieve repeatable CE-C$^4$D analyses of urinary MEL in shortest possible time and was set to 40 s at 950 mbar even though the 25 μm i.d. capillary was not completely filled with fresh BGE solution. The partial capillary flushing was sufficient for stable CE-C$^4$D performance as evidenced by repeatability data presented in Section 3.3. and ensured short analysis time. All measurements were performed at a constant voltage of +30 kV applied at the injection side of the separation capillary. Capillary temperature was maintained at 25 °C and injections were performed hydrodynamically at 50 mbar for 5 s (50 μm i.d. capillary) and at 50 mbar for 2 or 10 s (25 μm i.d. capillary). The CE instrument was controlled and analytical signals were acquired by ChemStation CE software.

## 3. Results and discussion

### 3.1. Optimization of BGE solution

MEL (see Fig. 1 for its structure) is a hydrazinium base with a predicted p$K_a$ value for carboxylic acid and the second nitrogen of 3.47 and 7.05, respectively (http://www.chemicalize.org, September 7, 2016). MEL can thus be separated in cationic form by