Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 889–894

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A microchip electrophoresis strategy with online labeling and chemiluminescence detection for simultaneous quantification of thiol drugs

Yong Huang[a,b], Shulin Zhao[a,*], Ming Shi[a], Hong Liang[a,*]

[a] Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (Ministry of Education), College of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin 541004, China
[b] College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China

ARTICLE INFO

Article history:
Received 13 October 2010
Received in revised form 2 March 2011
Accepted 3 March 2011
Available online 11 March 2011

Keywords:
Microchip electrophoresis
Chemiluminescence detection
Thiol drugs
Online labeling

ABSTRACT

An integrated microfluidic device with online labeling and chemiluminescence (CL) detection was developed for the simultaneous quantification of thiol drugs. In this device, the online labeling, electrophoresis separation and CL detection were compactly integrated onto a glass/poly(dimethylsiloxane) (PDMS) hybrid microfluidic chip. CL detection was based on the oxidation reaction of N-(4-aminobutyl)-N-ethylisoluminol (ABEI) and o-phthalaldehyde (OPA) labeled thiol drugs with NaBrO. Four thiol drugs including 2-mercaptopropionylglycine (2-MPG), captopril (CP), 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP) were employed as model compounds to examine the utility of the system. It was indicated that the separation and detection of four drugs can be completed within 90 s. Detection limits (S/N = 3) for the thiol drugs tested were in the range of 8.9×10^{-9}–13.5×10^{-9} M. The application of the present system was demonstrated by analyzing the thiol drugs in human plasma samples.

1. Introduction

Thiol-containing drugs are widely used for the treatment of many diseases. For example, D-penicillamine and 2-mercaptopropionylglycine (2-MPG) are frequently used as therapeutic drugs in Wilson's disease and rheumatoid arthritis, and as efficient antidotes in heavy-metal poisoning [1,2]. 6-Thioguanine (6-TG) and 6-mercaptopurine (6-MP) are used as anticancer drugs [3]. *N*-acetylcysteine was used as a mucolytic agent for the treatment of chronic bronchitis, and as an effective antidote for acetaminophen poisoning [4,5]. Other thiol-containing drugs such as captopril, mesna and thyreostats are also applied in different clinical fields. However, severe adverse reactions to oral thiol-drugs use have been described in subjects in which abrupt incremental dosing of the drugs were started. This suggests that monitoring of the concentrations of these drugs in biological fluids during disease therapies is warranted. Therefore, there is a need for the development of rapid, simple, and sensitive analytical method to measure these drugs.

Numerous analytical methods have been described for the quantification of thiol drugs, such as spectrophotometry [6,7], spectrofluorimetry [8,9], NMR spectrometry [10], Fourier transform infrared spectrometry [11], colorimetry [12], flow injection analysis (FIA) [13,14], and high-performance liquid chromatography (HPLC) [15,16]. Among these methods, HPLC is the most popular method for the analysis of thiol drugs. However, the assay is sometimes time-consuming, because a pre-treatment of the biological sample (such as solid-phase extraction or pre-column derivatization of analyte) is usually necessary prior to its injection into the instrument. In some cases complex gradient systems are also required, and long analytical times are needed due to prolonged elution times that add up to equilibration time between runs. Capillary electrophoresis (CE) coupled with various detection techniques such as UV, electrochemical and laser induced fluorescence (LIF) have also been developed for the determination of thiol drugs in biological samples [17,18].

Microchip electrophoresis (MCE) has been proven be a powerful separation technique for the analysis of chemical species [19]. Compared with conventional analytical techniques, MCE offers many advantages including extremely small sample size as low as nanoliters to femtoliters, high separation speed and efficiency, ease of integration and automatization. Chemiluminescence (CL) detection was one of the most sensitive detection techniques. MCE couple CL detection has become an attractive and alternative detection scheme for sensitive detection in MCE, and was successfully applied for the determination of metal ions [20], amino acids [21], catecholamines [22] and proteins [23]. Recently, our group developed an MCE–CL assay system for the analysis of biogenic amines in biological sample [24], and high detection sensitivity was achieved by pre-column derivatization.

* Corresponding authors at: College of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin 541004, China. Tel.: +86 773 5849646; fax: +86 773 5832294.
E-mail addresses: zhaoshulin001@163.com (S. Zhao), hliang@gxnu.edu.cn (H. Liang).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.03.007

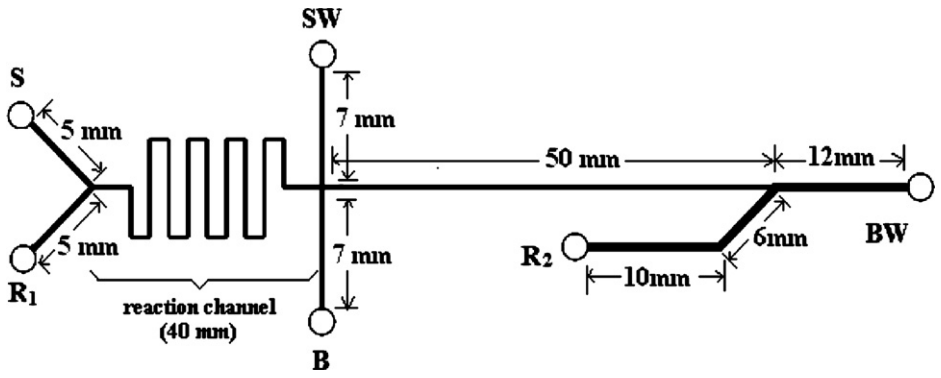

Fig. 1. The layout and dimensions of the glass/PDMS hybrid microchip used in this work. S, sample reservoir; R_1, labeling solution reservoir; B, electrophoretic buffer reservoir; SW, sample waste reservoir; R_2, oxidizer solution reservoir; BW, buffer waste reservoir.

In MCE, the microfluidic channels are extremely small, which affects negatively the detection sensitivity. Actually, the sensitivity for the MCE–CL methods reported previously was not impressive, the detection of limits was usually in the range from 10^{-6} to 10^{-7} M. Using CL labeling approach, the sensitivity for CL detection can be remarkably improved because no CL reagent was added to the running buffer, and therefore, the background of the CL detection was extremely low. The aim of this work was to develop a rapid, sensitive and selective MCE–CL system for the simultaneous quantification of thiol drugs in biological samples. Therefore, a new microfluidic device integrated with precolumn and postcolumn reactor was designed. Conditions for online labeling, electrophoresis separation and CL detection of the targeted analytes were studied, and the quantification of the thiol drugs in human plasma was demonstrated.

2. Experimental

2.1. Chemicals and solutions

Captopril (CP), 2-mercaptopropionylglycine (2-MPG), 6-thioguanine (6-TG), 6-mercaptopurine (6-MP) and o-phthalaldehyde (OPA) were provided by Sigma Chemicals (St. Louis, MO, USA). N-(4-aminobutyl)-N-ethylisoluminol (ABEI) was purchased from Fluka (Buchs, Switzerland). Sodium dodecyl sulfate (SDS) was obtained from Taopu Chemicals (Shanghai, China). All other chemicals used in this work were of analytical grade or better. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work. The electrophoretic buffer was 20 mM borate buffer (pH 9.6, adjusted with 1 M NaOH solution) containing 18 mM SDS. The oxidizer solution was 40 mM sodium carbonate buffer (pH 12.0, adjusted with 1 M NaOH solution) containing 1 mM NaBrO. The labeling solution was 0.1 mM ABEI and 0.05 mM OPA in electrophoretic buffer. Stock solutions of ABEI and OPA were prepared in methanol and diluted with 20 mM borate solution (pH 9.0). Stock solutions of thiol drugs were prepared in 20 mM borate solution (pH 9.6). All solutions were filtered through 0.22 μm membrane filters before use.

2.2. Apparatus and microfluidic chip

The assay was carried out using a laboratory built MCE–CL system as described previously [25]. The microchip assembly was mounted on the *X–Y* translational stage of an inverted microscope (Olympus CKX41) that also served as a platform of CL detection. CL signal was collected by means of a microscope objective. After passing a dichroic mirror and a lens, CL photons were detected by a photomultiplier (PMT, Hamamatsu R105). The PMT was mounted in an integrated detection module including HV power supply, voltage divider, and amplifier. The output signal of PMT was recorded and processed with a computer using a Chromatography Data System (Zhejiang University Star Information Technology, Hangzhou, China). A multi-terminal high voltage power supply, variable in the range of 0–8000 V (Shandong Normal University, Jinan, China), was used for sample injection and electrophoresis separation. The inverted microscope was placed in a black box.

A schematic layout of the glass/PDMS hybrid microchip is shown in Fig. 1. The width of all microchannels except oxidizer introduction channel (250 μm) is 70 μm; the depth of all microchannels is 25 μm. All reservoirs were 4.0 mm in diameter and 2.0 mm deep. The channel from reservoirs S and R_1 to the cross intersection was used for sampling and labeling, the channel between B and BW was used for the separation. The channel between the cross intersection and SW was used for delivering sample waste, and the channel between R_2 and BW was used for the oxidizer introduction. The join-point of the oxidizer introduction channel with the separation channel was used for the collection of CL.

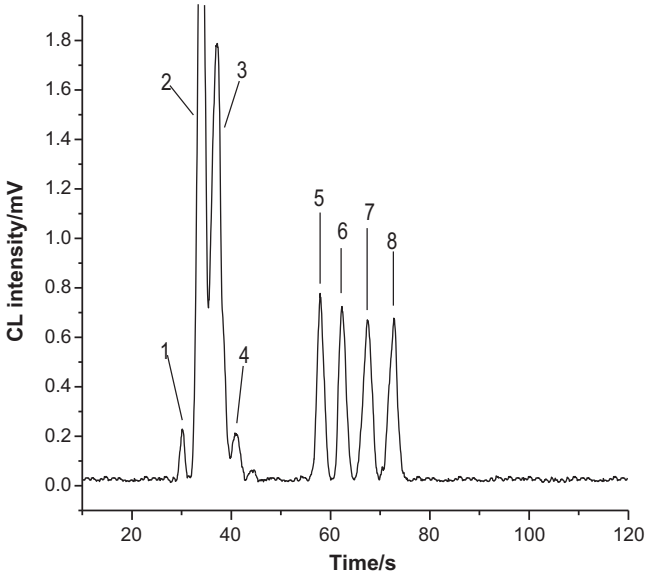

Fig. 2. Electropherogram obtained from the separation of a mixture of standard thiol drugs. Electrophoretic electrolyte was 20 mM borate buffer (pH 9.6) containing 18 mM SDS. The oxidizer solution was 40 mm sodium carbonate buffer (pH 12.0) containing 1 mM NaBrO. Labeling solution was 20 mM borate buffer (pH 9.6) containing 0.1 mM ABEI and 0.05 mM OPA. Potential applications for sample labeling, injection and separation were described in experimental section. All analytes concentrations are 4.0×10^{-7} M. Peaks: (1, 3, 4) decomposition product of tagging reagent; (2) ABEI; (5) 6-MP; (6) 6-TG; (7) 2-MPG; (8) CP.

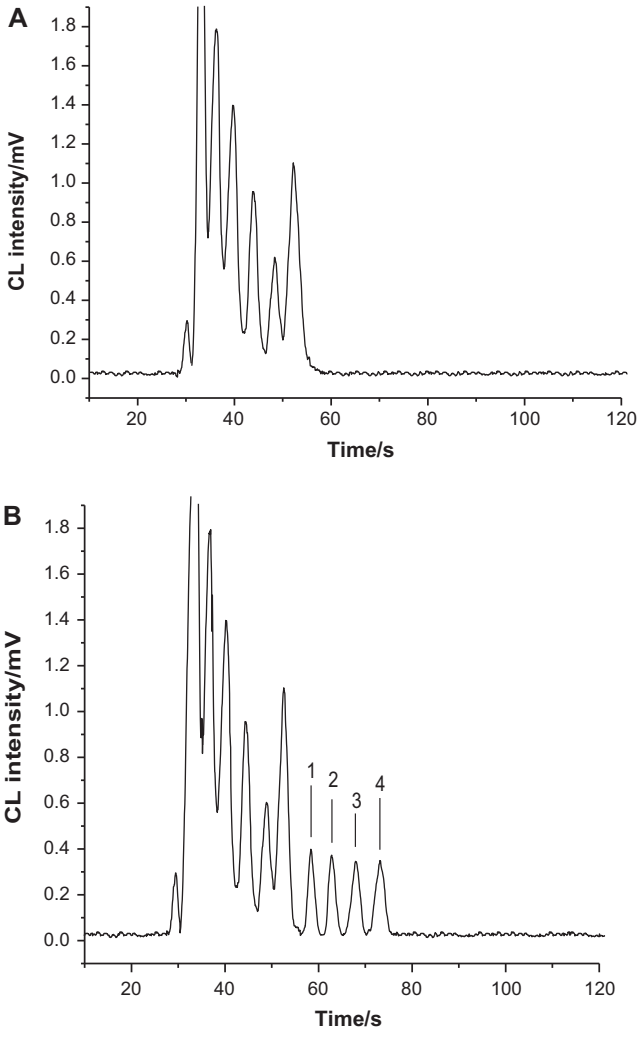

Fig. 3. Typical electropherograms obtained from the separation of a human plasma sample (A) and the sample spiked with four thiol drugs at 2.0×10^{-7} M each (B). The experimental conditions were as in Fig. 2. Peaks: (1) 6-MP; (2) 6-TG; (3) 2-MPG; (4) CP.

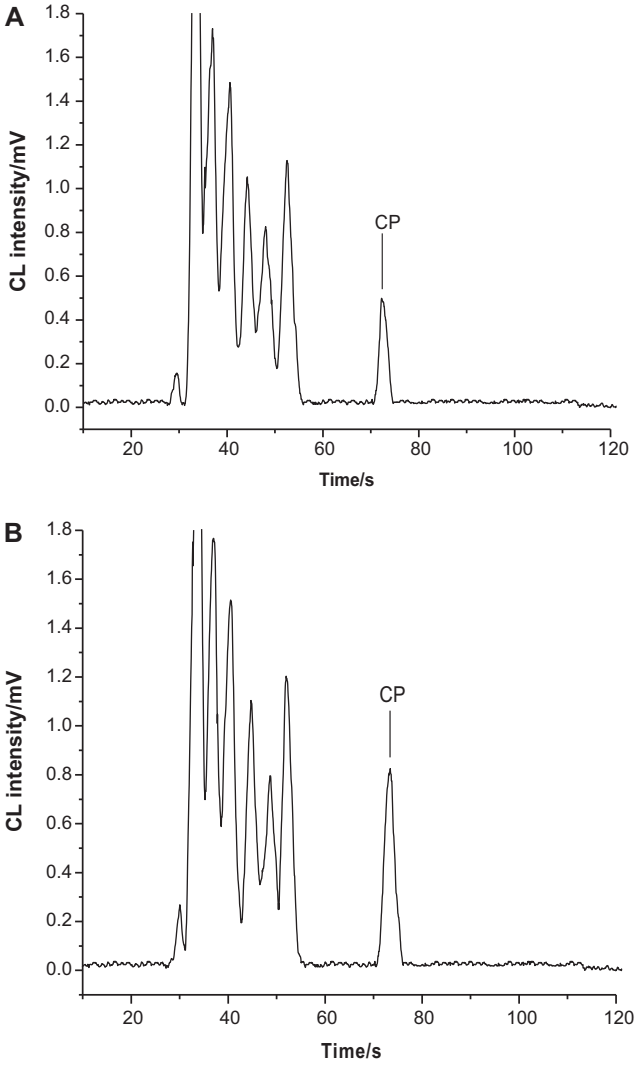

Fig. 4. Electropherograms obtained from the separation of a human plasma sample (collection at 3 h) from a healthy volunteer after administration of a single 100 mg CP oral dose (A) and the sample spiked with CP at 2.0×10^{-7} M (B). The experimental conditions were as in Fig. 2.

2.3. Sample preparation

The human plasma samples were obtained from healthy volunteer, which was stored at −20 °C until analyzed. A portion (400 μL) of a human plasma sample was diluted with ice-cold 800 μL of acetonitrile and shaken vigorously for 5 min to precipitate proteins. After centrifuging at 16,000 rpm for 10 min, the supernatant was transferred into a 1.5-ml vial and dried with a nitrogen stream. The residue was dissolved in 400 μL of 20 mM borate buffer at pH 9.6. Ninety microliters of this solution was spiked with 10 μL mixed solution of thiol drugs. Plasma samples of various contents of thiol drugs were similarly prepared by spiking the plasma with the desired amount of thiol drugs. For pharmacokinetic study of captopril, a healthy volunteer ingested 100 mg captopril. The plasma samples were collected from this individual at times 15, 30, 60, and 90 min, and 2, 2.5, 3, 4, and 6 h after oral administration. The preparation of these clinical plasma samples was similar to the above mentioned-procedure except no spiking.

2.4. Procedures for online labeling, separation and detection

The microchannels were rinsed with 1 M NaOH for 30 min before the first use. Between two consecutive runs, the microchannels were rinsed sequentially with 0.1 M NaOH, water and electrophoretic buffer for 2 min each. After reservoirs S, R_1, B, SW and BW were filled with the electrophoretic buffer, reservoir R_2 was filled with oxidizer solution, and vacuum was applied to reservoir BW in order to fill the separation channel with the electrophoretic buffer. Then, the electrophoretic buffer solution in reservoirs S and R_1 was replaced with sample solution and labeling solution, respectively. Platinum wires as electrodes were inserted into these reservoirs. Gated injection method [26] was employed in injecting labeled samples into the separation channel. During online labeling, a set of electrical potentials were applied to the reservoirs: reservoir R_1 at 1600 V, reservoir S at 1600 V, reservoir B at 2000 V, reservoir SW at 480 V, reservoir BW at 0 V, and reservoir R_2 at 550 V. In these voltage configurations, the sample and the labeling solution were electroosmotically pumped into the reaction chamber for derivatization, and the reactants and products into the separation channel were not allowed. After 10 s, potentials were switched to reservoirs S, R_1, SW, and BW at 1600, 1600, 480, and 0 V and B, R_2 floating for 2 s. In this step, the sample was injected into separation channel. Then the potentials at each reservoir were reapplied as: reservoir R_1 at 1600 V, reservoir S at 1600 V, reservoir B at 2000 V, reservoir SW at 480 V, reservoir BW at 0 V, and reservoir R_2 at 550 V for

Table 1
Calibration curves and detection limits for the determination of four thiol drugs by the proposed MCE–CL assay.

Thiols	Linear regression equation[a]	Linear range (10^{-8} M)	Correlation coefficient (r^2)	Detection limit (S/N = 3, 10^{-9} M)
6-MP	$H = 18.870C + 1.260$	2.0–400	0.9970	8.9
6-TG	$H = 17.613C + 0.354$	2.0–400	0.9944	10.0
2-MPG	$H = 16.483C - 1.280$	2.0–400	0.9918	11.7
CP	$H = 16.495C - 4.271$	2.0–400	0.9987	13.5

[a] H, peak height (relative CL intensity, μV); C, the concentration of the analyte (10^{-8} M).

MCE–CL detection. The sample was transported and separated in separation channel toward reservoir BW, and mixed with oxidizer solution from R_2 reservoir at the join-point of oxidizer introduction channel with the separation channel to produce visible light of maximum wavelength at 455 nm, which was detected by PMT.

3. Results and discussion

3.1. Optimization of online labeling conditions

Based on the rapid reaction between thiols with ABEI and OPA [27], an online labeling strategy was adopted in this study, and the reaction conditions such as concentration of ABEI and OPA, pH of labeling solution, and reaction time were optimized. Firstly, the effect of ABEI and OPA concentration on CL intensity was investigated. It was found that these thiol drugs showed all almost constant CL intensity when ABEI concentration above 0.1 mM and OPA concentration above 0.05 mM were used. Therefore, 0.1 mM ABEI and 0.05 mM OPA were selected as the optimized concentration for further studies. The pH of labeling solution affects also the CL intensity, the CL intensity of thiol drugs increased with the increase of labeling solution pH from 8.4 to 9.6, and the CL intensity reached constant after the pH value was greater than 9.6. Then, 9.6 was chosen as pH value of the labeling solution for the following experiments. The effect of sampling time on the CL intensity was also examined. The results indicated that the CL intensity of thiol drugs increased with increasing the sampling time, and kept constant after 10 s. Thus, 10 s of sampling time was used for each run.

3.2. Optimization of CL detection

To maximize the sensitivity of CL detection, the concentration of NaBrO and sodium carbonate, and pH of oxidizer solution were optimized. NaBrO as oxidizer in MCE–CL system affects obviously the CL intensity. The CL emission increased with the increase of NaBrO concentration, and kept almost constant after the concentrations were higher than 1.0 mM. Thus, 1 mM NaBrO solution was used in the following experiments. The effect of oxidizer solution pH on the CL intensity was examined in the range of 10.0–12.5. The results indicate that the CL signal first increased and then decreased with the increase of pH value. The maximum CL signal was observed when the pH value was 12.0. Because the chemiluminescent reaction is pH dependent. It can be seen from above the results that the oxidizer solution pH has evident influence on CL intensity. Therefore, 12.0 was chosen as pH value of the oxidizer solution for the following experiments. The effect of sodium carbonate concentration from 20 mM to 50 mM on the CL intensity was also investigated. The results indicate that maximal CL intensity for each analyte was observed when the concentration of sodium carbonate was 40 mM.

3.3. Optimization of separation conditions

In order to achieve high separation efficiency for the thiol drugs tested, a micellar electrokinetic chromatography (MEKC) separation mode was employed. The parameters affecting the separation such as concentration of SDS and borate, and pH value of electrophoretic buffer were optimized. It was found that the migration times and the resolution of four thiol drugs are lightly increases with the increase of borate buffer concentration from 10 mM to 35 mM. Furthermore, it was also noted that the separation efficiency for four thiol drugs increases with the augment of running buffer pH from 8.5 to 10.5, but four thiol drugs can still not separated completely under above conditions. In order to achieve an efficient separation, a popular surfactant, SDS, was selected as a micellar solution adding into running buffer, and the influence of SDS concentration range from 10 mM to 25 mM on the separation was investigated. The baseline resolution was observed with electrophoretic buffer containing SDS at 18 mM. On the basis of the above results, the optimum separation conditions were obtained as following: running buffer consisted of 20 mM borate and 18 mM SDS at pH 9.6. Under the optimized separation conditions, a mixture of standard thiol drugs was analyzed, and the electropherogram obtained is shown in Fig. 2.

3.4. Analytical figures of merit

The developed MCE–CL method was evaluated by in terms of the respond linearity, the limit of detection, and the reproducibility

Table 2
Precision and recovery for the determination of thiol drugs spiked in plasma samples.

Sample number	Thiols	Added (10^{-7} M)	Found (10^{-7} M)	Precision (RSD, %, $n = 5$)	Recovery (%)
1	2-MPG	0.6	0.57	3.6	95.0
	CP	0.6	0.56	2.5	93.3
	6-TG	0.6	0.58	2.1	96.7
	6-MP	0.6	0.57	3.3	95.0
2	2-MPG	3.0	3.04	2.8	101.3
	CP	3.0	2.89	3.9	96.3
	6-TG	3.0	2.85	2.3	95.0
	6-MP	3.0	2.83	2.1	94.3
3	2-MPG	10.0	9.20	3.7	92.0
	CP	10.0	9.72	4.3	97.2
	6-TG	10.0	9.54	2.2	95.4
	6-MP	10.0	9.36	3.6	93.6

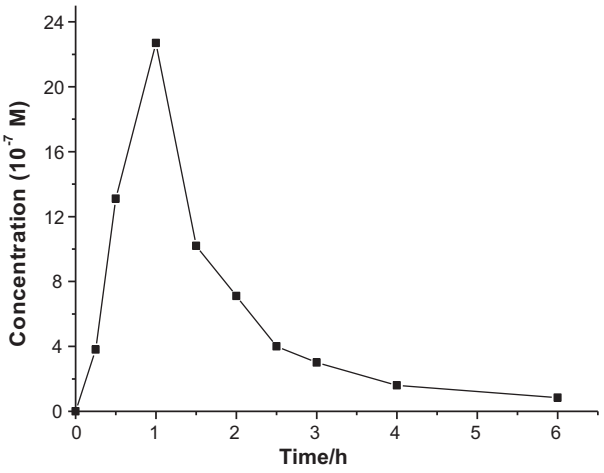

Fig. 5. Mean plasma CP concentration–time profiles after administration of a single 100 mg oral dose to a healthy volunteer.

(precision) of migration time and peak height. Under the optimized conditions, series concentrations of standard thiol drugs were analyzed to test the linearity, and each concentration was repeatedly tested 5 times. The results were summarized in Table 1. Calibration curves showed good linearity, the linearity ranges were from 2.0×10^{-8} to 4.0×10^{-6} M for all thiol drugs. Based on S/N = 3, detection limits for 6-MP, 6-TG, 2-MPG and CP were estimated to be 8.9 nM, 10.0 nM, 11.7 nM and 13.5 nM, respectively. The repeatability was tested by analyzing a mixture of standard thiol drugs at concentration of 6.0×10^{-7} M each for 9 times, and demonstrated by the relative standard deviations (RSDs). The RSDs for migration time and peak height were less than 3.3% and 4.6%, respectively.

3.5. Analysis of human plasma samples

The present MCE–CL method was applied for the quantification of thiol drugs in human plasma. First, a blank plasma sample from healthy volunteer was analyzed to examine the interference from endogenous compounds in human plasma. Fig. 3 shows the typical electropherograms obtained from a human serum sample without and with the thiol drugs. As can be seen from Fig. 3, no peak was observed across the migration time of thiol drugs in the electropherogram of blank plasma (Fig. 3A). After the plasma sample was spiked with thiol drugs at 2.0×10^{-7} M each, four peaks were observed in the electropherogram (Fig. 3B). The peaks for 6-MP, 6-TG, 2-MPG and CP were easily identified with a good match of retention time with the standards. This result indicated that none of the endogenous compounds in human plasma would interfere with the determination of the thiol drugs tested. The results obtained are summarized in Table 2. Further, the pharmacokinetics of CP was investigated in a healthy volunteer after a single oral dose administration of 100 mg CP and plasma samples were collected at interval, 0.25–6.0 h. Fig. 4A shows a typical electropherogram obtained from a human plasma sample collection at 3 h. Only one peak was observed across the migration time of thiol drugs, the peak was identified to be CP on the basis of its relative migration time and adding CP standard solution (as shown in Fig. 4B). In these plasma samples, CP was detected. The plasma level of CP and its time-dependence are shown in Fig. 5. Recoveries of CP in these plasma samples spiked with CP were also studied. The results indicated that the recoveries were in the range of 94.8–100.3%.

4. Conclusion

A MCE–CL system with online labeling has been developed for simultaneous quantification of thiol drugs. ABEI coupled OPA showed promising properties as the CL tagging reagents for online labeling and electrophoretic separation in terms of simple operation, fast reaction and high detection sensitivity. The separation and detection of four thiol drugs including 2-MPG, CP, 6-TG and 6-MP need only 90 s. Detection limits were in the range of 8.9×10^{-9}–13.5×10^{-9} M. The applicability of the present system was demonstrated by the quantification of thiol drugs in human plasma samples with good precision and reproducibility. The developed MCE–CL method has the advantages of simplicity, low cost, short analysis time, high selectivity and sensitivity. Further, this system promises to be useful for the determination of other thiol drugs in complex matrix.

Acknowledgments

Financial support from the National Natural Science Foundations of China (No. 20875019), the National Basic Research Program of China (No. 2010CB534911), and the Natural Science Foundations of Guangxi Province (No. 0991012Z, 2010GXNSFF013001) is gratefully acknowledged.

References

[1] A. Członkowska, J. Gajda, M. Rodo, Effects of long treatment in Wilson's disease with D-penicillamine and zinc sulphate, J. Neurol. 243 (1996) 269–273.
[2] A. Zinellu, C. Carru, S. Sotgia, L. Deiana, Plasma D-penicillamine redox state evaluation by capillary electrophoresis with laser-induced fluorescence, J. Chromatogr. B 803 (2004) 299–304.
[3] M.V. Relling, M.L. Hancock, J.M. Boyett, C.H. Pui, W.E. Evans, Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia, Blood 93 (1999) 2817–2823.
[4] T. Toyo'oka, Recent advances in separation and detection methods for thiol compounds in biological samples, J. Chromatogr. B 877 (2009) 3318–3330.
[5] L.F. Prescott, J.A. Critchley, The treatment of acetaminophen poisoning, Ann. Rev. Pharmacol. Toxicol. 23 (1983) 87–101.
[6] B.G.T. Corominas, J. Pferzschner, M.C. Icardo, L.L. Zamora, J.M. Calatayud, In situ generation of Co(II) by use of a solid-phase reactor in an FIA assembly for the spectrophotometric determination of penicillamine, J. Pharm. Biomed. Anal. 39 (2005) 281–284.
[7] F.E.O. Suliman, H.A.J. Al-Lawati, S.M.Z. Al-Kindy, I.E.M. Nour, S.B. Salama, A sequential injection spectrophotometric method for the determination of penicillamine in pharmaceutical products by complexation with iron(III) in acidic media, Talanta 61 (2003) 221–231.
[8] A.A. Al-Majed, Specific spectrofluorometric quantification of D-penicillamine in bulk and dosage forms after derivatization with 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole, Anal. Chim. Acta 408 (2000) 169–175.
[9] J. Russell, J.A. Mckeown, C. Hensman, W.E. Smith, J. Reglinski, HPLC determination of biologically active thiols using pre-column derivatisation with 5,5′-dithio-(bis-2-nitrobenzoic acid), J. Pharm. Biomed. Anal. 15 (1997) 1757–1763.
[10] J. Nelson, Nuclear magnetic resonance spectroscopic method for determination of penicillamine in capsules, J. Assoc. Off. Anal. Chem. 64 (1981) 1174–1178.
[11] M. Zeeb, M.R. Ganjali, P. Norouzi, S.R. Moeinossadat, Selective determination of penicillamine by on-line vapor-phase generation combined with Fourier transform infrared spectrometry, Talanta 78 (2009) 584–589.
[12] A.M. Pimenta, A.N. Araujo, M.C.B.S.M. Montenegro, Sequential injection analysis of captopril based on colorimetric and potentiometric detection, Anal. Chim. Acta 438 (2001) 31–38.
[13] Z.D. Zhang, W.R.G. Baeyens, X.R. Zhang, G.V. Weken, Chemiluminescence flow-injection analysis of captopril applying a sensitized rhodamine 6G method, J. Pharm. Biomed. Anal. 14 (1996) 939–945.
[14] P. Viñas, I. Lopez Garcia, J.A. Martinez Gil, Determination of thiol-containing drugs by chemiluminescence—flow injection analysis, J. Pharm. Biomed. Anal. 11 (1993) 15–20.
[15] K. Kuśmierek, G. Chwatko, R. Głowacki, E. Bald, Determination of endogenous thiols and thiol drugs in urine by HPLC with ultraviolet detection, J. Chromatogr. B 887 (2009) 3300–3308.
[16] A.F. Hawwa, J.S. Millership, P.S. Collier, J.C. McElnay, Development and validation of an HPLC method for the rapid and simultaneous determination of 6-mercaptopurine and four of its metabolites in plasma and red blood cells, J. Pharm. Biomed. Anal. 49 (2009) 401–409.
[17] X. Yang, H. Yuan, C. Wang, X. Su, L. Hu, D. Xiao, Determination of penicillamine in pharmaceuticals and human plasma by capillary electrophoresis with

in-column fiber optics light-emitting diode induced fluorescence detection, J. Pharm. Biomed. Anal. 45 (2007) 362–366.
[18] P. Lochman, T. Adam, D. Friedecký, E. Hlídková, Z. Škopková, High-throughput capillary electrophoretic method for determination of total aminothiols in plasma and urine, Electrophoresis 24 (2003) 1200–1207.
[19] D. Mark, S. Haeberle, G. Roth, F. Stetten, R. Zengerle, Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications, Chem. Soc. Rev. 39 (2010) 1153–1182.
[20] T. Nogami, M. Hashimoto, K. Tsukagoshi, Metal ion analysis using microchip CE with chemiluminescence detection based on 1,10-phenanthroline–hydrogen peroxide reaction, J. Sep. Sci. 32 (2009) 408–412.
[21] M. Hashimoto, K. Tsukagoshi, R. Nakajima, K. Kondo, A. Arai, Microchip capillary electrophoresis using on-line chemiluminescence detection, J. Chromatogr. A 867 (2000) 271–279.
[22] R. Su, J.M. Lin, F. Qu, Z. Chen, Y. Gao, M. Yamada, Capillary electrophoresis microchip coupled with on-line chemiluminescence detection, Anal. Chim. Acta 508 (2004) 11–15.
[23] X. Huang, J. Ren, On-line chemiluminescence detection for isoelectric focusing of heme proteins on microchips, Electrophoresis 26 (2005) 3595–3601.
[24] S. Zhao, Y. Huang, M. Shi, Y.M. Liu, Quantification of biogenic amines by microchip electrophoresis with chemiluminescence detection, J. Chromatogr. A 1216 (2009) 5155–5159.
[25] S. Zhao, X. Li, Y.M. Liu, Integrated microfluidic system with chemiluminescence detection for single cell analysis after intracellular labeling, Anal. Chem. 81 (2009) 3873–3878.
[26] K.W. Ro, K. Lim, H. Kim, J.H. Hahn, Poly(dimethylsiloxane) microchip for precolumn reaction and micellar electrokinetic chromatography of biogenic amines, Electrophoresis 23 (2002) 1129–1137.
[27] A. Sano, H. Nakamura, Chemiluminescence detection of thiols by high-performance liquid chromatography using *o*-phthalaldehyde and *N*-(4-aminobutyl)-*N*-ethylisoluminol as precolumn derivatization reagents, Anal. Sci. 14 (1998) 731–735.